JOURNAL OF GENETICS

Volume XX. 1928–29

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No. 1 (July, 1928)

No. 2 (November, 1928)

CORRIGENDA IN VOL. XX

p. 75, under column "$F_2 ex F_1 \times$ d. cream" in mating (6) for "15:0:17:0" read "0:15:16:1."

p. 309, line 14, for "*Ibid.*" read "Journ. Gen."

THE GENETICS OF WHEAT SPECIES CROSSES. I.

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(With One Plate and Five Text-figures.)

CONTENTS.

INTRODUCTION.

THE history of these investigations goes back to 1921, when a genetic analysis of an F_2 generation from *T. turgidum* × *T. vulgare* was attempted. Of the new types that appeared in this F_2 one of the most striking was the one now usually known as speltoid. Special attention was paid to it, partly because its distinctive features suggested that it would provide favourable material for discovering how new characters arose in the F_2, and partly because it appeared to be associated with the high chromosome number of the *vulgare* parent. To understand the reason for this, and other similar associations, seemed important.

These problems were finally solved in 1926 and the conclusions have already been published[16]. It was found that the keeled glume of *turgidum* and the round glume of *vulgare* were associated with other characters such as glume thickness and ear density, that these characters were all due to a single pair of factors **K** and **k**, and that speltoids were simply **KK**-*vulgare* plants, thus:

vulgare ... ... = 42-chromosome-**kk**   speltoid = 42-**KK**
round-glumed *turgidum* (extracted from the
   turgidum × *vulgare* cross) ... ... = 28-chromosome-**kk**   *turgidum* = 28-**KK**

so that from the cross *turgidum* × *vulgare* we obtain the two new types, speltoid and round-glumed *turgidum*.

But if we examine the four types in question it is by no means obvious
from their appearance that the two 42-chromosome types differ in the
same factor as the two 28-chromosome types (Pl. I, figs. *b, c*; *e, f*), and at
this stage the work proceeded in two directions, as described in this
paper. The first was a morphological study of the exact effect of **K**. The
second was concerned with the relation of speltoid to other wheat types.
It was found that the glume and rachis characters of round-glumed
vulgare, speltoid and *Spelta*—the three most distinct 42-chromosome
types—were due to three factors **k**, **K** and **K$_s$**, which form a series of
multiple allelomorphs, or more probably consist of groups of completely
linked factors showing a similar relationship. But since **K**, the central
term of this series, is the factor that gives the distinctive keel found in
most members of the 28-chromosome wheat group, the question arose
whether the other factors in the series also occur in this group; and
if so, whether these three factors alone, or other factors in the same
series, are sufficient to account for all the principal glume and rachis
types found in the two groups. It will be shown that very probably
they are.

Later in the paper the question whether **k**, **K** and **K$_s$** should be
regarded each as a single factor or as a group of closely linked factors is
discussed; but as it is not yet possible to decide this finally the former
view is adopted for simplicity in the earlier sections.

The morphological studies were based on a detailed examination of
the following forms: *T. dicoccum* var. Ajar; *T. turgidum* var. Rivet;
round-glumed *turgidum* extracted from the cross Swedish Iron × Rivet;
T. persicum Vav. var. Persian Black; *T. Spelta* vars. Grey Spelt and
White Spelt; a speltoid form extracted from the cross Swedish Iron
× Rivet; *T. vulgare* var. Swedish Iron.

They were confirmed by examining a large number of types of the
species *dicoccum, turgidum, durum, vulgare* and *Spelta*, as far as this
could be done without dissecting the ears[1].

In the various crosses to which reference is made the parent forms
were those named above, and also *T. durum* var. Indian Runner and
T. vulgare var. Yeoman.

Except for the recognition of *T. persicum* Vav., I have followed the
classification of the genus given by Percival in his monograph (12).

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[1] I am indebted to Professor Percival for giving me facilities, on several occasions, for
examining his magnificent collection of wheats from all parts of the world; but I have not
yet had time to study all these forms in relation to the characters dealt with in this
paper.

THE TYPES OF THE 42-CHROMOSOME GROUP.

The group has been divided into the following species: *T. vulgare* Host., *T. sphaerococcum* Perciv., *T. compactum* Host. and *T. Spelta* L.

The last-named species is sharply differentiated from the rest by its tough, keeled glumes firmly investing the grain, by its brittle rachis and very lax ear. With the possible exception of a few forms of *vulgare* to be mentioned later, the species other than *Spelta* are characterised by a tough rachis, an ear of variable density but nearly always much denser than that of *Spelta*, and loose glumes that are rounded at the base or weakly keeled. They are easily separated from each other by other characters, but all are closely allied. The other form to be considered is speltoid, which resembles *Spelta* in its tough, sharply keeled glumes and lax ear, but has a tough rachis. This form is not cultivated, but arises occasionally as a mutant from *vulgare*, to which it is similar in all but the characters mentioned. It will be shown that the three glume and rachis types—*vulgare*[1], speltoid and *Spelta*—are due to three multiple allelomorphs, **k**, **K** and **K_s**. The relation is not expressed by *vulgare* = **sV**, speltoid = **sv**, *Spelta* = **SV**, as has been supposed (6, 17).

Other workers have dealt with the relation between *Spelta* and *vulgare* (6, 8, 11), and as my own results are in agreement it is only necessary to give a brief account. F_2 from *vulgare* × *Spelta* gave a ratio of 83 vulgare : 163 intermediate : 91 *Spelta*, or nearly 1 : 2 : 1. The heterozygotes fluctuate from almost like *vulgare* to almost like *Spelta*, and the character differences determined by **k** and **K_s** are the above-mentioned differences between *Spelta* and *vulgare* type-forms. From *Spelta* the heterozygotes can be distinguished by the fact that their glumes can be pulled away from the grains somewhat more easily; from *vulgare* the separation may be more difficult, but can be effected on the basis of a weak development of the collar at the base of the glume, slight toughness of glume, and somewhat flatter faced glume. In all heterozygotes the *Spelta* characters are most highly developed towards the top of the ear, and least at the bottom. The most complete study of the cross has been made by Nilsson-Leissner (11), who has continued the investigation to F_4. He concludes that only a single factor is involved, but that in the heterozygotes the "degree of spelting" depends on several independent modifying factors, which primarily affect the laxity of the ear both in the heterozygous and the homozygous forms; the laxer the

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[1] Here, and in later sections of this paper, I refer only to the round-glumed varieties. These, and the weakly keeled forms, make up the bulk of the species. The question is dealt with more fully in the conclusion.

ear, the more *Spelta*-like the plant. Leighty and Boshnakian[8] give evidence for the existence of a two-factor difference in some *Spelta* × *vulgare* crosses, but Nilsson-Leissner has criticised this conclusion.

It has been shown[16] that the speltoids extracted from the cross *vulgare* × *turgidum* differ from *vulgare* by a single factor **K**. Here again a 1 : 2 : 1 ratio is obtained; the intermediates fluctuate from almost like speltoid to almost like *vulgare*, and the speltoid characters are most developed at the top of the ear. The normal type of speltoid mutant studied by Nilsson-Ehle[10] also differs from *vulgare* by one factor; as is well known the other mutants give irregular ratios and have been shown by Winge[17] to exhibit irregular chromosome behaviour.

To show that, with the three types in question, we are dealing with a series of multiple allelomorphs, and not with multiple factors, several lines of evidence were open. The cross speltoid × *Spelta*, which would complete the case, has not been made; but the cross *turgidum* × *Spelta* should serve equally well, as we shall see, to show that **K** is allelomorphic to **K$_s$**. Further it will be shown that, morphologically, *vulgare*, speltoid and *Spelta* form a graded series; and finally (p. 19) that **K** and **K$_s$** probably show the same linkage value with the factor for awns.

To recapitulate briefly the conclusions of an earlier paper[16], it was found that the formula for *turgidum* is 28-chromosome-**KK**, for *vulgare* 42-chromosome-**kk**, and for speltoid 42-chromosome-**KK**—**K** and **k** being carried by chromosomes that pair and segregate normally in the species hybrid; while the glume difference between *turgidum* and speltoid is due to the extra *vulgare* chromosomes. Hence, if **K$_s$** is allelomorphic to the speltoid factor **K**, the cross *turgidum* × *Spelta* (42-chromosome-**K$_s$K$_s$**) should give no round-glumed (**kk**) forms; and this was found to be the case. A more detailed description of the results of this cross will be given on a later page (p. 10), but it may be stated here that we do actually get all the types that would be expected on the assumption that **K** and **K$_s$** are allelomorphs, and no others.

The morphological comparison between the types *vulgare*, speltoid and *Spelta* will be given in detail since later in the paper a comparison will be made between these three types and the corresponding types of the 28-chromosome group. The characters dealt with are all governed by the factorial series we are discussing and are:—the keel of the glume and the development of the secondary nerves, the thickness of the glume, the collar at its base, its shape, the density of the ear, and the structure of the rachis. If we pass up the series from *vulgare*, through speltoid to *Spelta*, we find that the following changes occur:

(1) An increased thickness of the glume, illustrated by the transverse sections shown in Text-fig. 1 *a–c* and the longitudinal sections shown in Text-fig. 2 *a–c*. The greatest increase occurs towards the base of the glume, and in *Spelta* is carried further up than in speltoid. Here, as with some of the other characters, transverse sections do not alone form a basis for comparison, since the thickness varies at different levels up the glume.

(2) The keel is greatly developed; it is broader in *Spelta* than in speltoid, but this may be the result of the increase in thickness of the glume (see Text-fig. 1 *a–c*). In wheat the keel is not equally developed all the way up the glume; the figures are drawn from glumes cut transversely across the centre, at which point the development is least.

(3) The lateral nerves are weak but variable in *vulgare*, and highly developed in speltoid (Text-fig. 1 and Plate I, fig. *f*). In *Spelta* they are clearly visible on the face, but in most forms (as in Text-fig. 1 *c*) they are not visible in a section cut across the centre because the increase in thickness of the whole glume has included a filling-in of the spaces between the lateral nerves.

(4) The collar at the base of the glume is almost absent in *vulgare* and is well marked in speltoid (Text-fig. 5 *e, f*; p. 14). In *Spelta* it is again very well developed but its upper limit is made less conspicuous by the great increase in thickness of the glume itself just above the collar, which, however, stands out very clearly from the rachis below it.

(5) The face of the glume becomes progressively flatter. This can be seen in the longitudinal sections (Text-fig. 2 *a–c*) but cannot be shown adequately by figures.

(6) Speltoid is laxer in the ear than *vulgare*, but *Spelta* shows little additional increase (Plate I, figs. *e, f, g*). Nilsson-Leissner (11), comparing *Spelta* with speltoids that have originated as mutants, speaks of the former as distinctly more lax; but I have extracted very lax speltoids from species crosses. Laxity of ear is undoubtedly affected by a number of factors, and, after comparing (by eye) several *Spelta* forms with my speltoid types, I feel uncertain whether any increase in laxity is necessarily associated with the factor K_s, *i.e.* with the *Spelta* type.

(7) The rachis becomes progressively thinner in longitudinal section. This is most marked just above the points where the spikelets are attached. There is less change from speltoid to *Spelta* than from *vulgare* to speltoid.

(8) *Vulgare* has a tough rachis. In speltoid it is also tough, except that if the upper portions of the ear are rubbed fairly gently the rachis
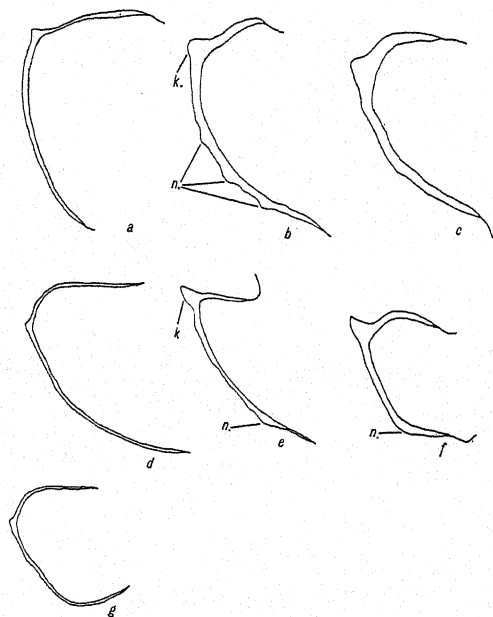
Text-fig. 1. Transverse sections through centres of glumes. (a) Round-glumed *vulgare*, (b) speltoid, (c) *Spelta*, (d) round-glumed *turgidum*, (e) *turgidum*, (f) *dicoccum*, (g) *persicum*. Drawings made with the aid of camera lucida. k. = keel, n. = lateral nerve. × 7.
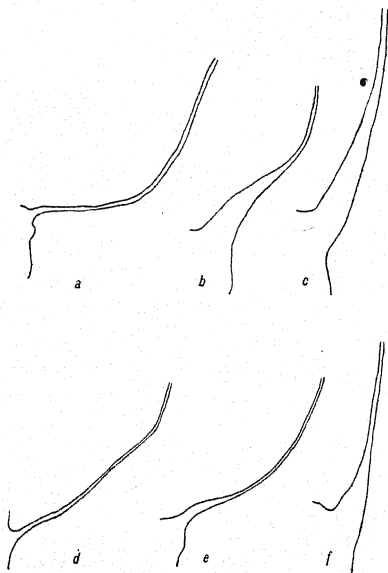
Text-fig. 2. Longitudinal sections through glumes, showing lower portions only. Lettering as in Text-fig. 1. Camera lucida drawings. × 7.

breaks in the manner typical of *Spelta*: that is it breaks just below the spikelet, so that each spikelet carries the portion of the rachis that comes above it (Text-fig. 3 *a*, and Text-fig. 4 *b*). If the lower part of the ear is rubbed hard the rachis is sometimes torn off just above the spikelet, where the rachis is rather thin, so that the spikelet carries with it the portion of the rachis that lies below it, as in *dicoccum*. In *Spelta* the rachis is brittle, and readily breaks in the way described—just below each spikelet. But as the rachis is very thin just above the spikelet it will break at this point fairly easily if prevented from breaking at the
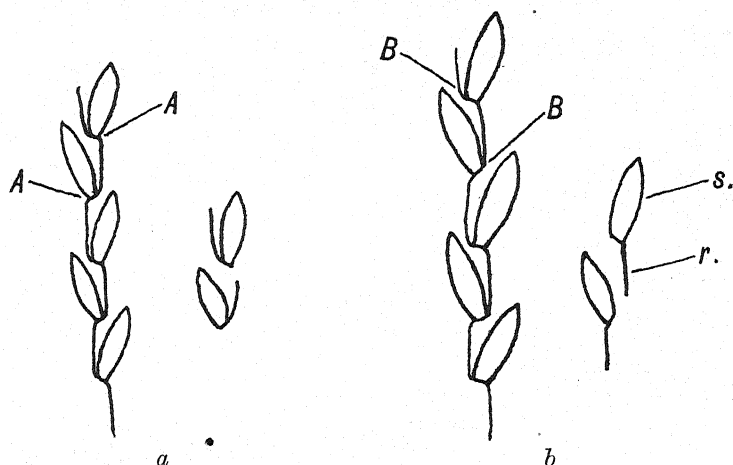
Text-fig. 3. Diagrammatic. (*a*) *T. Spelta.* (*b*) *T. dicoccum.* In *Spelta* the rachis breaks below the spikelet at *A*; in *dicoccum* above at *B*. *s.* = spikelet, *r.* = rachis.

usual place; that is it breaks in the *dicoccum* manner, viz. at *B* (Text-fig. 3 *b*) instead of at *A* (Text-fig. 3 *a*). Evidently in the three 42-chromosome types there is an increase in weakness of the rachis both above and below the spikelet as we go from **k** to $\mathbf{K_s}$; but in this group the weakness becomes greatest just above the spikelet and fracture occurs here when it does occur. It should be mentioned that brittleness of the rachis does not develop to its full in *Spelta* until the ear is ripe, and the character of the rachis in other 42-chromosome wheats depends to some extent upon external conditions. An interesting example of this was seen in the case of an American variety known as Marquillo. This is a *vulgare* wheat with a slightly keeled glume; it is not suited to conditions at Cambridge, where it suffers severely from attacks of *Puccinia glumarum*,

and the rachis has a marked tendency to break like that of *Spelta*, a tendency which it does not show when grown in America[1].

Evidently *Spelta* is characterised by an intensification of some of the features that distinguish speltoid from *vulgare*. This is seen in (1) thickness of glume, (5) a flatter faced glume, (7) structure of rachis and (8) brittleness of rachis; it is less clear in (2) the keel, (3) lateral nerves, (4) collar of the glume, or (6) ear density. I think it may be taken that this agrees with the supposition that the three types are due to a series of three multiple allelomorphs. The four characters in which an increase is less evident are difficult to judge exactly; nor in my opinion is an increased development of all the characters a necessary consequence of the suggested relationship.

Nilsson-Leissner looks upon the speltoid mutants as diluted *Spelta* types, in agreement with my description, though he expresses the genetic relationship somewhat differently to accord both with his own observations and with the theory of Winge (17). Winge's theory of the origin of speltoid mutants demands that *T. vulgare*, being a hexaploid species, should contain in its gametes three similar sets of 7 chromosomes each instead of 21 different ones; and, considering only one chromosome from each set of 7, he writes the haploid formula of *vulgare ABC* and of speltoid *ABB*, where *A*, *B* and *C* denote similar but not identical chromosomes. Nilsson-Leissner writes the factors in these chromosomes as follows:

A	B	C		A	B	C		A	B	B
	L_1 l_2	s			l_1 L_2	S			l_1 L_2	l_1 L_2
	Spelta				*Vulgare*				Speltoid	
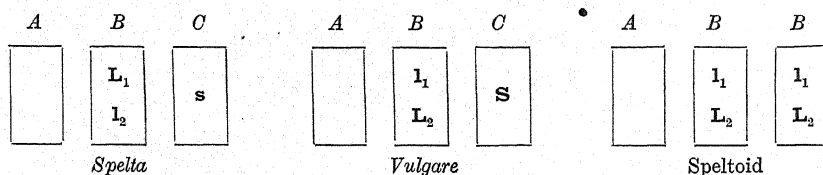
His **s** is my K_s, and his **S** my **k**. L_1 and L_2, etc. are factors which affect the laxity of the ear and, agreeably with his conclusion mentioned above, give a speltoid (diluted *Spelta*) type if enough of them are present. If Winge's views are accepted—they are considered again on p. 19—my view would differ from Nilsson-Leissner's in that I should suppose *B* to carry **K**, allelomorphic to **k** and K_s, instead of L_1 and L_2. I am not quite clear what results to expect, on Nilsson-Leissner's theory, from the cross speltoid × *Spelta*. Assuming classification to be possible it would

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[1] I am indebted to Mr Olaf S. Aamodt, of the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, Washington, for sending me ears of Marquillo grown under American conditions.

on my view probably give a ratio of 1 speltoid : 2 intermediate : 1 *Spelta*. There is however the possibility that the F_1 would breed true to the intermediate condition, giving in F_2 and later generations neither of the parental types but a range of intermediates that breed true. This possibility will not be considered further here as it would lead us too far from our present subject, but it is hoped to discuss the matter in detail at an early opportunity. In the meantime reference may be made to Darlington (2).

As already stated, I have taken in this paper the view that **k**, **K** and **K$_s$** are related as a multiple allelomorphic series, and this is confirmed by their relation to the factor for awns, given on a later page. Difficulties in the way of expressing with certainty the relation between the types are considered in the conclusion.

The Types of the 28-chromosome Group.

Some of the species in this group are not very well separated, and it is not certain that the present classification is final. The following are the species that have been recognised by one or other of the two leading authorities, Percival and Vavilov: *T. dicoccum* Schübl. and its wild form *T. dicoccoides* Körn., *T. orientale* Perciv., *T. durum* Desf., *T. polonicum* L., *T. turgidum* L., *T. pyramidale* Perciv. and *T. persicum* Vav.

With regard to the glume and rachis characters we can recognise three main types:

(1) With keeled tough glumes and brittle rachis, the rachis breaking just above the spikelet, instead of just below as in *Spelta* (Text-fig. 3). All forms of *dicoccoides* and some of *dicoccum*[1] fall into this class. The first of these will not be considered further as it is separated from *dicoccum* by characters other than those we are considering.

(2) With keeled, loose glumes, and a tough rachis. This description applies to all forms of *orientale, polonicum, durum, turgidum* and *pyramidale* (but see later, p. 23, for a fuller discussion), and without doubt these species must be regarded as closely allied. Some forms of *dicoccum* also fall into this class. *Polonicum* is readily distinguished by its very long papery glumes, and some associated characters, which are all due to a single factor difference from *durum* or *turgidum*, as shown by Biffen (1) and Engledow (3). *Durum* and *turgidum* are not always easily separated, but this can usually be done by the character of the grain and the hairs on the young leaves. Since they are alike in their glumes

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[1] *T. dicoccum* Schrk. is absolutely defined by these characters. *T. dicoccum* Schübl., the classification adopted by Percival (12), includes in addition a number of forms with tough rachis and loose glumes.

they will be considered here together. *Orientale* and *pyramidale* are two species recently made by Percival[12]; the former is close to *dicoccum* and *durum* and the latter to *turgidum*.

(3) With rounded, loose glumes and a tough rachis = *T. persicum* Vav.

Hereafter in this paper the first of these three types will be referred to as *dicoccum*, the second as *turgidum* or *durum*, and the third as *persicum*.

Just as we have three principal glume and rachis types in the 42-chromosome group—*vulgare* ($\mathbf{kk}$), speltoid ($\mathbf{KK}$) and *Spelta* ($\mathbf{K_sK_s}$)—so have we three main types in the 28-chromosome group, and the types in the two groups are to a large extent parallel. Now speltoid differs from *vulgare* by the factor $\mathbf{K}$ that gives the keel to the glume of *turgidum*: the latter species is 28-chromosome-$\mathbf{KK}$, *vulgare* is 42-$\mathbf{kk}$, and speltoid 42-$\mathbf{KK}$. This suggests the question, what is the type 28-$\mathbf{K_sK_s}$? Further, could we identify this with *dicoccum* and embrace all the types within a scheme involving only three factors in a single multiple allelomorphic series? The obvious difficulty is that although *dicoccum* and *Spelta* both have tough glumes and a brittle rachis, they differ morphologically in that the rachis of the former breaks just above the spikelet, and that of the latter just below. Nevertheless, this solution has a great deal of evidence in its favour and the following formulae are tentatively suggested for the six types found in the cultivated wheats of the second (28-chromosome) and third (42-chromosome) groups:

vulgare $=42$-chromosome-$\mathbf{kk}$	speltoid $=42$-$\mathbf{KK}$ *Spelta* $=42$-$\mathbf{K_sK_s}$
persicum $=28$-$\mathbf{kk}+$ some other change	$\left.\begin{array}{l}\textit{turgidum}\\ \textit{durum}\end{array}\right\} =28$-$\mathbf{KK}$ *dicoccum* $=28$-$\mathbf{K_sK_s}$

To establish this will take at least three years' breeding work. But three lines of evidence now available strongly suggest the identity of the *Spelta* and *dicoccum* factors, and will be considered fully. The first was provided by a cross between *turgidum* and *Spelta* which I had to hand; the second by a comparison of the morphology of the six types; the third by examining the literature on crosses between various species of the second and third groups.

If we assume, as explained earlier (p. 4), that $\mathbf{K}$ is allelomorphic to $\mathbf{K_s}$ then the expectation from *turgidum* (28-$\mathbf{KK}$) $\times$ *Spelta* (42-$\mathbf{K_sK_s}$) is as follows:

(1) *turgidum*—28-$\mathbf{KK}$,

and transition forms, with intermediate chromosome number, to

(2) speltoid—42-$\mathbf{KK}$;

(3) the new type 28-$\mathbf{K_sK_s}$,

and transition forms, as before, to

    (4) *Spelta*—42-K_sK_s;

    (5) the form 28-KK_s,

and transition forms to

    (6) speltoid-*Spelta* heterozygote—42-KK_s.

It will be clear that no accurate classification of such an F_2 can be expected; especially on account of the various transition forms that may be expected to be associated with a chromosome number intermediate between 28 and 42. Such was the case, but there was no question that the expected types were all present, and no others. An F_2 of 145 plants was examined. The types *turgidum*, speltoid, *Spelta*, with intermediates and heterozygotes, were found as expected and need no comment. In
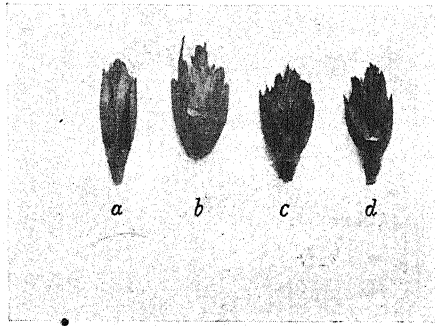
Text-fig. 4. Single spikelets to show breaking of the rachis: (*a*) *T. dicoccum*, carrying the portion of the rachis that lay below the spikelet; (*b*) *T. Spelta*, carrying the portion of the rachis that lay above the spikelet; (*c*) spikelet of a plant obtained from the cross *Spelta* × *turgidum*, and breaking like *dicoccum*; (*d*) spikelet from another plant from the cross *Spelta* × *turgidum*; ears from this plant broke in some places like *dicoccum* and in others like *Spelta* and the spikelet shown carries the portion of the rachis from above, like *Spelta*, as well as the portion of the rachis from below, like *dicoccum*. All natural size.

addition were *turgidum* forms with tough glumes suggesting *dicoccum* in shape, and a rachis that broke like *dicoccum* (Text-fig. 4 *c*). These forms were numerous: they were not occasional appearances that might be due to some unexpected irregularity. Presumably this is the expected form 28-K_sK_s; and apparently the *Spelta* factor K_s produces the *dicoccum* character when transferred to a 28-chromosome species. Confirmation was provided by the existence of transitional forms between *Spelta* and this *dicoccum*-like *turgidum*. These have a brittle rachis, but when an ear is rubbed out it breaks in parts like *dicoccum* and in parts like *Spelta*, in a more or less random manner. From one and the same ear one

therefore obtains spikelets carrying a portion of the rachis like *dicoccum*, and spikelets carrying a portion in the manner of *Spelta* (Text-fig. 4 *d*). In parenthesis, it should be mentioned that the *dicoccum*-like forms were clearly *turgidum* with this typically *dicoccum* character: the two species differ by other characters besides the tough glumes and brittle rachis. Only one possible disturbing feature was noticed in the cross, the significance of which is uncertain: there seemed to be less than the expected number of plants with a tough rachis.

The evidence from this cross is not perhaps as complete as could be wished; but it clearly supports strongly the view I have put forward, and no more definite conclusion could be hoped for.

Further evidence was sought by examining the results described by various authors for other crosses between the second and third groups of wheat species. The theory we are considering demands the following results:

(1) *Dicoccum* (28-K_sK_s) × *vulgare* (42-kk) should give the two new types *Spelta* and round-glumed *dicoccum*, and no others.

(2) *Dicoccum* × *Spelta* (42-K_sK_s) should give only *Spelta*, *dicoccum* and transition types.

(3) *Turgidum* and *durum* (28-KK) × *vulgare* should give two new types, namely speltoid and round-glumed *turgidum* or *durum*, and no others. That this is so appears from my own observations both on *turgidum* × *vulgare* and on *durum* × *vulgare*, and from those of other workers.

(4) *Turgidum* and *durum* × *Spelta*. This has been described and agrees with expectation. The new type *dicoccum* appears.

On the whole this expectation is borne out remarkably well; though considerable caution has to be used in interpreting many of the results that have been reported at one time or another. The crosses in question show various irregularities owing to the parental differences in chromosome number, and no critical analysis has previously been attempted. Usually the results have only been described in general terms; and though we can infer, since wheat species are most often diagnosed by the type of glume and rachis, that the description "*durum*" probably means a 28-chromosome wheat with a strongly keeled glume, we cannot always be sure of this. It is only rarely that particular characters are described accurately. This difficulty is most apparent in the earlier papers, since classification of the wheat forms has only approached finality in recent years. It is fairly certain, for instance, that in some cases round-glumed 28-chromosome plants extracted from an intergroup cross have been described as *vulgare* simply because they have round

glumes. Again it is probable that some authors have called speltoids
T. Spelta or "*Spelt*-like types." Finally, it has been shown that natural
crossing occurs fairly frequently in partially sterile wheat hybrids, and
the new forms sometimes noted as occurring in a species cross may
possibly have originated in this way. It seems unnecessary therefore to
deal thoroughly with all the crosses that have been described, and
mention will only be made of two papers. Of these the results of
Kajanus(7) are valuable; and this author actually expresses the belief
that *Spelta* and *dicoccum* owe their tough glumes and brittle rachis to
the same factor, exactly in agreement with my own conclusion. From
the cross *dicoccum* × *vulgare* he obtained two new types: *Spelta,* and a
form he calls "compressum" described as "*vulgare*-ähnliche Formen mit
stark abgeplatteten Ähren und gedrängten Ährchen." From this
description it is clear that his type compressum is the round-glumed
dicoccum that should segregate from the cross (see later, p. 14, for
description of round-glumed *turgidum* extracted from *turgidum* × *vulgare*
crosses). This therefore agrees exactly with our expectation given in (1)
above. In an earlier paper he actually shows that this synthetically pro-
duced *Spelta* behaves genetically like true *Spelta* when crossed with
vulgare(6). Malinowski(9) reports that *dicoccum* × *vulgare* gives the types
dicoccum, durum, vulgare and *Spelta*. The occurrence of Spelta agrees
with expectation, but the *durum* forms were not predicted above in out-
lining the expectation from this cross—their origin will be referred to
in a later paper. Malinowski also states that, as we expected in (2) above,
the cross *Spelta* × *dicoccum* gives nothing but *Spelta* and *dicoccum* forms.

On the whole there is no doubt that the results of other workers
agree well with the scheme here put forward. It is hoped to carry out
critical tests during the course of the next few years.

We may now consider the final line of evidence—a morphological
comparison of the types. The 42-chromosome types—*vulgare* (**kk**),
speltoid (**KK**) and *Spelta* (K_sK_s)—have been described already (p. 5).
It remains to compare the corresponding 28-chromosome types—round-
glumed *turgidum* (**kk**) extracted from a *turgidum* × *vulgare* cross,
turgidum (**KK**) and *dicoccum* (K_sK_s); and to see that the change in the
direction $k \to K \to K_s$ is the same in the two groups. The relation of the
round-glumed 28-chromosome *T. persicum* to the other species will be con-
sidered later. The characters controlled by the factorial series in question
will be given in the same order as before (p. 5). It will be realised of
course that we cannot show that the characters are affected to the same
degree in the two groups, only that they are affected in the same way.

(1) In the round-glumed *turgidum* the glumes are very thin and papery in texture; the progressive increase in thickness in *turgidum* and *dicoccum* is apparent in the figures (Text-figs. 1 and 2), and, as before, occurs chiefly near the base.

(2) The change from **k** to **K** gives the keel; while in *dicoccum*, as in *Spelta*, the keel is broader (Text-fig. 1); but when a large series of *turgidum*, *durum* and *dicoccum* forms is examined it becomes doubtful whether any increased development of the keel is associated with the *dicoccum* character.
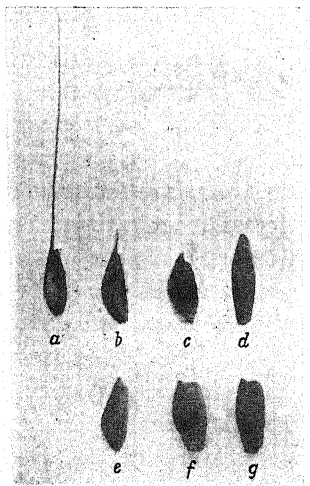
Text-fig. 5. One glume from each of the ears shown in Plate I. (*a*) *T. persicum* var. "Persian Black," (*b*) round-glumed *turgidum* extracted from the cross "Swedish Iron" × "Rivet," (*c*) *T. turgidum* var. "Rivet," (*d*) *T. dicoccum* var. "Ajar," (*e*) *T. vulgare* var. "Swedish Iron," (*f*) speltoid extracted from the cross "Swedish Iron" × "Rivet," (*g*) *T. Spelta* var. "White Spelt." All natural size.

(3) The influence on the lateral nerves is less obvious; partly because in thin glumes the nerves may be rendered more obvious by the reduction in thickness of the tissue lying between them (compare p. 5). In Rivet, the variety of *turgidum* used in the crosses, the lateral nerve is more prominent than in the extracted round-glumed *turgidum*, but the degree of development varies in different *turgidum* varieties. In *dicoccum* the lateral nerves are usually strong, but here again there may be considerable variation, and in addition the nerves may be obscured by the increased thickness of the glume itself. On the whole it is probable that the change we are considering does cause a greater development of the lateral nerves, but this is not certain.

(4) There is no collar in the round-glumed *turgidum*, and in consequence of this and of the fact that the glumes are very thin the latter are easily detached from the rachis. In *turgidum* the collar is well marked. In *dicoccum*, as in *Spelta*, the collar is strong and well developed but is obscured along its upper margin by the great thickness of the glume just above it. It is, however, nearly always defined (as in *Spelta*) by the fact that it is formed of different tissue from the glume, and is therefore of a slightly different colour; also it stands out very clearly where it joins the rachis.

(5) The glume of round-glumed *turgidum* is very rounded on the face, and that of *turgidum* much flatter; in *dicoccum*, as judged by a longitudinal section it is still flatter, and in transverse section it is very flat between the keel and the secondary nerve (Text-figs. 1 and 2).

(6) The ear of round-glumed *turgidum* is very. dense and thereby easily distinguished from that of *turgidum*, which is only moderately dense; in *dicoccum* there is no marked increase (Plate I, figs. *b–d*).

(7) The rachis becomes progressively thinner in longitudinal section, especially just above the points where the spikelets are attached. This is most marked in the change from *turgidum* to *dicoccum*, and in this differs from the parallel change in the *vulgare* group.

(8) The only brittle rachis form is *dicoccum*; here the fracturing of the rachis just above the spikelets is largely due to the extreme thinness of the rachis at these points, and this has been dealt with as character (7). If the rachis is prevented from breaking above the spikelet it will break just below, at any rate in some forms, and near the top of the ear; but this brittleness is probably not great enough to suggest much analogy with *Spelta*.

From this description we see that the change from **k** to **K** is of the same nature as in the 42-chromosome group, and the difficulty experienced in recognising this is due to the original differences between round-glumed *turgidum* and *vulgare*. It is possible that the intensity with which **K** acts differs in the two cases, but when we have completed our discussion of the relation between the types it will be realised that this might quite reasonably be expected.

The change from *turgidum* to *dicoccum* is certainly similar to that from speltoid to *Spelta* in characters (1), (4), (5) and (7); probably also in characters (2), (3) and (6), where there is no marked change in either case. But there is still some difficulty over the brittle rachis. In both groups the change in the direction $k \to K \to K_S$ brings about an increasing weakness of the rachis just above the spikelet, which is only more extreme

in *dicoccum* than in *Spelta*. But in *dicoccum* there is no great weakness below the spikelet; presumably its development in *Spelta* is in some way connected with the extra *vulgare* chromosomes, but exactly how is uncertain.

It remains to consider the only round-glumed 28-chromosome species, *T. persicum*. Crossed with *vulgare* this form gives only round-glumed types in F_2. It resembles round-glumed *turgidum* rather closely in its glumes (see Text-figs. 1 *d, g*; 2 *d, g*; 5 *a, b*), which in both types are rounded on the face, have very little keel (see especially the transverse sections in Text-fig. 1 *d, g*), are thin and papery, and are easily detached from the rachis. But *persicum* has a lax ear and very slender rachis, while round-glumed *turgidum* has a very dense ear and broad rachis, so that we cannot consider *persicum* as simply a 28-chromosome-**kk** form. The question is discussed more fully later on, but we may say here that it seems likely that **K** represents a group of two or more linked factors, and that in *persicum* this linkage has been broken.

THE EFFECT OF THE EXTRA CHROMOSOMES OF THE *VULGARE* GROUP.

Having described fully the types of the two groups we may turn to consider the change in glume shape and rachis type brought about by the increase in chromosome number from 28 to 42, and see if any factorial scheme can be suggested to account for the change. It was pointed out in an earlier paper[15] that this change is similar to that produced by the factor **K** itself, and it was therefore suggested[16] that the extra chromosomes actually carried a factor **K′** similar to or identical with **K**, the formula for *turgidum* being **KK** and for *vulgare* **kkK′K′**. We shall consider this possibility first of all by returning to the eight characters affected by **K**; the effect of the extra chromosomes being found by comparing *turgidum* (28-**KK**) with speltoid (42-**KK**), and round-glumed *turgidum* (28-**kk**) with *vulgare* (42-**kk**).

It will be seen that the extra chromosomes affect these eight characters, considered in the same order as before, much as the factor **K** does:

(1) For *turgidum* and speltoid, compare Text-figs. 1 *b* and *e*, and 2 *b* and *e*, and note the increased thickness of the glume. The glumes of both *vulgare* and round-glumed *turgidum* are thin and the difference is not readily seen in the drawings; to the touch, however, the latter is much thinner and more papery than the former.

(2) The keel of speltoid is broader than that of *turgidum* (Text-fig. 1 *b* and *e*) but not otherwise more developed. *Vulgare* may have slightly

more keel than round-glumed *turgidum* (Text-fig. 2 *a* and *d*) but in both the keel is weak.

(3) The lateral nerves of speltoid are more prominent than those of *turgidum* (cf. Text-figs. 1 and 2).

(4) The collar at the base of the glume is more marked in speltoid than in *turgidum*. Neither of the round-glumed forms has a noticeable collar; but the glumes of round-glumed *turgidum* are more easily detached than those of *vulgare*, and this is probably a comparable feature since the presence of a collar makes the glumes more difficult to remove.

(5) The face of the glume is flatter in speltoid than in *turgidum* (compare Text-figs. 1 *b* and *e*, 2 *b* and *e*). Both round-glumed *turgidum* and *vulgare* are rounded in the lower half, but in the latter the glume is flatter in the upper half than it is in the former.

(6) Speltoid has a laxer ear than *turgidum* (Plate I, figs. *c* and *f*) and *vulgare* a laxer ear than round-glumed *turgidum* (Plate I, figs. *b* and *e*).

(7) Seen in longitudinal section the rachis of *vulgare* is thinner than that of round-glumed *turgidum*, and of speltoid thinner than that of *turgidum*.

(8) It should be noted that although all four forms may be classed as having a tough rachis, yet speltoid shows some tendency to brittleness near the top of the spike and *turgidum* shows none.

Evidence of a similar nature was given in an earlier paper[15] where a comparison was made between two groups of plants obtained from the cross ♀ *vulgare* × ♂ F_2 (*vulgare* × *turgidum*). The two groups are distinguished by chromosome number, the first group containing plants with 35 chromosomes and having the formula **Kk** or **kk**, the second with the same factorial formula but having an addition of at least three of the extra *vulgare* chromosomes and usually from five to seven, or a total chromosome number of from 38 to 42. In comparing these two groups, whether we compared **kk** plants or **Kk** plants, it was found that the effect of the extra *vulgare* chromosomes was to increase the thickness of the glume, to make the face of the glume flatter, and to increase the laxity of the ear; while among the **Kk** plants we could also observe an increased development of the lateral nerves and of the collar at the base of the glume. Again, at one time or another plants of the following composition have been obtained: 35-chromosome-**KK** and 28-**KK**; 35-**Kk** and 28-**Kk**; 35-**kk** and 28-**kk**; and comparison of these types once more leads to the conclusion that the extra chromosomes have a similar effect to that of the factor **K**. We cannot in every case quoted observe an effect on all the eight characters, for in some cases only three or four may be

affected; but when all the cases are considered there is no question that the extra chromosomes increase the development of all the eight characters. Thus, if we compare the types 35-**Kk** and 28-**Kk** an increase in thickness of glume is not very apparent, but is very striking when 35-**KK** is compared with 28-**KK**.

Finally, the F_1 from speltoid × *vulgare* is intermediate, but closer to speltoid than to *vulgare*; and in F_2 the heterozygotes, though fluctuating widely, are probably nearer on the average to speltoid. In the 42-chromosome group therefore there is no question of complete dominance either of **K** or **k**, but possibly **K** should be regarded as the dominant. In the 28-chromosome group on the other hand **Kk** plants approach the round-glumed (**kk**) form; so much so that sometimes the two types are difficult to separate. But this agrees with the view here expressed. **Kk** may be nearer to **kk** than to **KK**; but if the extra chromosomes carry an additional factor **K′**, it is not surprising to learn that the heterozygous speltoid (**Kk K′K′**) is nearer to speltoid (**KK K′K′**) than to *vulgare* (**kk K′K′**)[1].

For these reasons it has been concluded that, in all probability, the extra *vulgare* chromosomes do actually carry a factor **K′** similar to the factor **K**. In support of this ·is the conclusion reached in an earlier paper(16) that the inheritance of wax on the foliage must be explained by giving *turgidum* the formula **WW** and *vulgare* the formula **ww W′W′**, where **W** and **W′** are two similar factors. If this be accepted we can write the genetic formulae of all the glume and rachis types dealt with in this paper as follows:

persicum = (**kk**) + some other change	*turgidum* } = (**KK**)	*dicoccum* = (**K$_s$K$_s$**)
vulgare = (**kk**) **K′K′**	*durum* }	
	speltoid = (**KK**) **K′K′**	*Spelta* = (**K$_s$K$_s$**) **K′K′**

where factors enclosed in brackets are carried by homologous chromosomes.

Since the species of the *dicoccum* group are tetraploid we might expect each to contain two pairs of factors in the **K** series; and the species of the *vulgare* group each to contain three. So far this third pair of factors has not been revealed; but, if it exists, we can conclude that *turgidum* and *vulgare* both possess the same pair(16), or at any rate that they differ by two factors that are very close together in the same series of multiple

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[1] These, and other, considerations make the question of representation difficult. I have called the round-glumed form **k** because it seems to be the one in which characters are absent, and have looked upon **K$_s$** as the highest term in the series. If, however, the round-glumed form proves to be dominant this representation should be reversed.

allelomorphs. When other intergroup crosses have been closely analysed it may be possible to identify them, and of course it is always possible that all species of the second and third groups are the same in this respect.

This section cannot be concluded without referring again to the now well-known theory put forward by Winge[17] to account for the origin of speltoid mutants. It demands that *vulgare* should have the formula **BB CC** and speltoid the formula **BB BB**. Now if the factor I have called **K'** is actually the same as the factor **K**, the formulae I have given for *vulgare* and speltoid become **kk KK** and **KK KK** respectively; agreeing exactly with Winge's formulae. I was at first inclined to believe that **K** and **K'** were identical, and since Winge reached his conclusion from a cytological study of the origin of speltoids as mutants from *T. vulgare*, and mine was obtained from a genetic study of their origin from a species cross, it seemed at first that the conclusions must be regarded as confirming each other in striking fashion. But now that the above comparison of the types has been done more thoroughly it seems to me that we are not justified in going so far: indeed it is obvious that on the evidence given **K'** might easily be **K$_s$**, or any other term in the series if other terms exist, and we are unable to identify **K'** more exactly by the methods so far used. If we do identify the factors **K'** and **K$_s$** we must suppose, to agree with Winge's theory, that the third factor in *vulgare*, mentioned above as not yet discovered, is the factor **K**.

Nilsson-Ehle, to whom much of our knowledge of the genetic behaviour of speltoid mutants is due, does not believe[10] that Winge's theory accounts adequately for the origin of all the types he has found, since some of these involve a change in only a single factor or at any rate in less than a whole chromosome. It is hoped to consider these questions in more detail elsewhere.

THE LINKAGE BETWEEN **K** AND THE FACTOR FOR AWNS.

The evidence so far given that the factors **k**, **K** and **K$_s$** form a multiple allelomorphic series, though strong, cannot be regarded as complete. Fortunately confirmation of this or a closely similar relationship is given by the fact that they show the same linkage value with the factor for awns. In the same way we can confirm the previous conclusion[16] that speltoid differs from *vulgare* by the same factor that gives the keel to *turgidum*; since the cross bearded *turgidum* × beardless *vulgare* gives the same linkage value between the factors for awns and for glume keel as is

found between awns and speltoid in the cross beardless speltoid × bearded *vulgare*.

Bearded (**bb**) and beardless (**BB**) are usually stated as differing by a single factor with beardless dominant. This result applies to crosses between fully bearded wheats and the common type of so-called beardless wheats which have short tips to the paleae, sometimes two centimetres or more long at the top of the ear. The Howards[5] describe quite beardless wheats which differ by two factors from bearded forms. I shall refer here only to the former, tipped, type. Although there seems no doubt that the difference in question is really due to a single factor, and this result has been reported on numerous occasions, most crosses give in F_2 a deficiency of bearded plants. This is usually small, and according to Kajanus[6] is due to lessened hardiness of the bearded type. I am not convinced that this is always the explanation but shall not discuss the question further here: the deficiency has to be mentioned however as it makes it difficult to be sure of the exact value of the linkage between **B** and **K**.

Linkage between **B** and the factor for the Spelta ear type, which I have called **K$_s$**, has been reported before; thus Kajanus[6] gives a coupling of $1 : 2 : 2 : 1$, equivalent to 33 per cent. of crossing over; while Nilsson-Leissner[11] gives 35 per cent. My own figures, given below, agree best with a crossing-over value of 28 per cent.

Bearded *Spelta* × beardless *vulgare* F_2:

	Spelta		Heterozygous *Spelta*		*Vulgare*	
	Bearded	Beardless	Bearded	Beardless	Bearded	Beardless
Found	39	52	24	135	8	73
Expectation with 28 % crossing over	43	40	33	132	6·5	76

The lack of agreement between observation and expectation is due to the deficiency of the bearded class.

Nilsson-Ehle[10] gives a coupling value of $1 : 2·8$ in the case of the cross beardless speltoid × bearded *vulgare*. Agreement between observation and expectation is again not very good (the exact figures need not be given here), owing to a deficiency of the bearded class equal to 2·7 times the standard error. Nevertheless the linkage value found by Nilsson-Ehle, which is equivalent to 26 per cent. crossing over, agrees very well with the one I found for the *Spelta* × *vulgare* cross. When we consider that these two cases are, perhaps, the only certain cases of linkage known in wheat, it leaves us with practically no doubt that the

relation between **k**, **K** and **K$_s$** must be of the nature of a series of multiple allelomorphs[1].

It remains to consider the linkage between keeled and bearded in the cross *turgidum* × *vulgare*. Genetic results with this cross have been given elsewhere (16), but the inheritance of awns was not described as the data then available pointed to the possibility that some undiscovered irregularity might be at work. It will be enough for our present purpose to say that the cross (*turgidum* × *vulgare*) F_1 ♀ × *turgidum* ♂, in which there is no disturbance from sterility, has given a ratio of 33 beardless : 36 bearded, very nearly the expected 1 : 1 for a single factor difference. Classified also for keeled and round the same cross gave 13 beardless keeled : 22 bearded keeled : 20 beardless round : 14 bearded round. The numbers are small, but clearly suggest the expected linkage between bearded (**b**) and keeled (**K**), and give a value of about 39 per cent. crossing over with a standard error of about 6 per cent. This value does not, it is true, agree any too closely with the 26 per cent. of crossing over found by Nilsson-Ehle for the factors **b** and **K** in the cross beardless speltoid × bearded *vulgare*; but the discrepancy may well be due to the small numbers in the *turgidum* × *vulgare* back cross, and to the uncertainty that arises in Nilsson-Ehle's cross from the deficiency of bearded plants. As we have already said, the fact that linkage exists is itself strong evidence since no other certain case is known in wheat[1]. It is also possible that in a cross between two species differing in chromosome number, as do *turgidum* and *vulgare*, crossing over may not occur with the same frequency as in a cross between two varieties with the same chromosome number.

CONCLUSION.

Before concluding this paper we may discuss more fully the question whether the factors **k**, **K** and **K$_s$** really represent single factors or groups of completely linked factors. In some ways the latter view seems the more probable. It is unusual to find a single factor controlling the development of so many characters as have been attributed to these, and there is evidence that each may represent at least two completely linked factors. It seems possible that the keel of the glume, the development of the secondary nerves, and the collar at the base, may be due to one factor only; and it is possible, but less likely, that the same factor controls the thickness of the glume and whether it should be flat or

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[1] My attention has since been called to another case of linkage, between colour and hairiness of chaff, given by Kajanus (6).

rounded on the face. But it is far less likely that the rachis characters should be controlled by this same factor as well. This is supported by the case of *persicum*, which agrees on the whole with round-glumed *turgidum* in its glume characters, but not in its rachis characters. This result may be due to a modifying factor in *persicum*, but it is more likely that **k** represents at least two completely linked factors, and that the two forms in question differ by one of these. It should be stated, however, that the two forms are not identical in their glume characters (see Text-fig. 5); apart from the well developed secondary tooth of *persicum*, which is no doubt due to a separate factor, the glumes of this form, though fragile, are not detached from the rachis quite so easily as those of round-glumed *turgidum*.

Cases of complete linkage are probably not uncommon in Cereal crosses. Thus Biffen[1] and Engledow[3] found that in crosses between *T. polonicum* and *T. durum* the distinctive features of the former—the long grain, long glume, and some other glume characters—were all due to a single factor **P**. Yet a race of *polonicum* with long glumes and short grains does exist[1], and it would appear therefore that **P** represents at least two factors of which one has been changed to give the short grained race. No crosses have as yet been made to test this point. In *Hordeum*, too, cases of complete linkage seem to exist[4], though there are complicating features here. It may well be significant that in all these instances—the "factors" **k**, **K** and **K$_s$**, *T. polonicum*, and *Hordeum*—the characters showing linkage have some claim to be regarded as specific distinctions.

If it be agreed that **K** and its allelomorphs represent groups of factors, the further question arises to what extent the suggested multiple allelomorph relation holds, but this is difficult to answer. The difference between **K** and **K$_s$**, for example, might be due to the addition of a modifying or intensifying factor to the linkage group. Nevertheless, there is little doubt that the morphological difference between speltoid and *Spelta* is in some respects, if not in all, a quantitative one, and it is therefore quite likely that if **K** really does represent a group of factors then **K$_s$** represents a similar group in which one or more of the former have been changed in a quantitative manner. But, once again, a decisive answer cannot be given.

To see the genetic results given in this paper in their true perspective it is necessary to say more about the variation within the genus of the characters in question. In the past, classification was based principally on the glume; *vulgare* and the related *compactum*, for example, were

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[1] I am indebted to Professor Percival for bringing this race to my notice.

regarded as having a round glume and for this reason "Persian Black," a variety of the species now known as *T. persicum* Vav., was at one time classed as *T. vulgare*, to which it is in fact quite unrelated. There is, I think, no question that the shape of the glume is extremely important for classifying wheats, but it is now well known that the presence or absence of a keel is not alone of much importance. In *vulgare* round loose glumed forms are common, and of the others many have only a weakly developed keel; but in a few forms the keel is fairly strong, and, as we have seen, is well developed in speltoids. Many of the weakly keeled forms seem to fall into a fairly well defined class and have, in addition to the weak keel, glumes that invest the grains a little more closely, a slight collar at the base of the glume, and a moderately lax ear. They suggest in fact that between **K** and **k** there may exist another group of factors **K**$_1$, close to **k**, but no crosses have been made to test this possibility, and there is no question that this type is so near the round-glumed type that an F_2 would be impossible to classify since the heterozygote is almost certain to be intermediate. Again, a few varieties, such as the Australian "Cedar," have glumes that are much tougher than those of the class just described, and perhaps a rather stronger keel. There is thus no question that there are a number of forms intermediate between round-glumed *vulgare* and speltoid, and the genetic relation of these intermediates to the latter types is quite unknown; indeed it cannot yet be said how many types exist, but it is probably significant that in the forms I have examined there is a clear tendency for development of keel and toughness of glume to go together, as would be expected from the genetic evidence on the types that have been studied. The results of Nilsson-Leissner[11] should be of value here.

Turning now to the 28-chromosome group we find a similar state of affairs. It was stated (p. 9) that all forms of *orientale, polonicum, durum, turgidum* and *pyramidale* have loose, keeled glumes, and a tough rachis. This statement needs the qualification that the keel is not always developed to the same extent; and in some forms, especially in *durum*, it may be weak. In *dicoccum* also, as described by Percival, grades of keels almost down to the round-glumed *persicum* can be found. As in the *vulgare* group, these various types of keel have not yet been properly classified, and no genetic evidence is available as to their relation to the other types we have dealt with.

For these reasons it is premature to discuss the systematics of the genus in the light of the genetic results presented. Investigation of the glume and rachis characters was begun by the writer with the belief that

they had a more definite value in classification than they are now known to have, and it was hoped that knowledge of their genetic relation might give valuable information on the course of evolution in the genus. Nevertheless it cannot be doubted that the characters in question have an important relation to classification. Several examples can easily be quoted. Thus, the *dicoccum* and *Spelta* types are confined to their respective species in the two different groups. A strongly keeled glume characterises the majority of forms of the 28-chromosome group and is found in the 42-chromosome group only in the type speltoid, which has probably originated from *vulgare* in the manner suggested by Winge; moreover this type is distinguished by a group of characters which do not exist together in the 28-chromosome group. Finally a round glume is common in the *vulgare* group, but in the other group is found in a few forms only. In these circumstances it seems probable that, even if it be premature at the present stage to build conclusions on the results given, yet a thorough study of variation in the characters we have been discussing and a thorough knowledge of their inheritance should help us to understand the genetic relation and origin of the wheat species. The factorial system put forward here is not yet finally proved; but though it may need modification it undoubtedly gives a simple explanation for a large number of complicated facts, and it probably indicates the direction along which a solution is most likely to be found. The fact that so many types exist, and that segregation of groups of completely linked factors probably occurs, points the need for caution in formulating the true genetic relation between the types investigated.

Vavilov has pointed out (13) that related species show parallel series of variations. In the two wheat groups we have been considering it is interesting to see that we have two series of variations—from round-glumed *turgidum* to *dicoccum* and from *vulgare* to *Spelta*—which are not quite parallel but are nevertheless due to the same factor changes: from k to K and K_s in each case. Owing to some fundamental genetic difference, in this case the extra chromosomes of the *vulgare* group, the effect of a factor may appear to be different in different species. It seems probable here, to give the most striking example, that K_s brings out in the *vulgare* group a character which it does not produce in the *dicoccum* group, viz. fragility of the rachis just below each spikelet. This conclusion may be of general interest, and encourages the hope that in other genera, as in wheat, variation in allied species may be represented by the same genetic changes even in cases where this does not at first sight seem possible.

SUMMARY.

Important characters in classifying wheats are the presence or absence of a keel on the glume, whether or not the glumes are tough and invest the grains closely, and whether the rachis is brittle or tough; the characters are closely associated. Within the second (28-chromosome) and third (42-chromosome) groups there is a considerable range of variation in these characters, but the following forms may be taken as typical:

(a) 42-chromosome group.

(1) With round, loose glumes, and tough rachis; common in *vulgare*, and here referred to as *vulgare* type.

(2) With keeled, tough glumes, tough rachis and lax ear; the type speltoid.

(3) With keeled, very tough glumes, brittle rachis and lax ear; characterising *T. Spelta*.

(b) 28-chromosome group.

(4) With round, very loose glumes, and tough rachis; found only in *T. persicum* Vav.

(5) With keeled, loose glumes, and tough rachis; found in most forms of *turgidum* and *durum*, and some other species, and here referred to as the *turgidum* type.

(6) With keeled, tough glumes, and brittle rachis which breaks above each spikelet instead of below as in *Spelta*; found only in *dicoccum* and the wild *dicoccoides*, and here referred to as the *dicoccum* type.

Types (1), (2) and (3) may be classed as **kk**, **KK** and $\mathbf{K_S K_S}$ where **k**, **K** and $\mathbf{K_S}$ represent three factors in a series of multiple allelomorphs, or three groups of completely linked factors having a similar genetic relationship; the latter view is the more probable.

Types (4), (5) and (6) are related to each other by the same factors as types (1), (2) and (3); except that in type (4), *persicum*, the factor in the group **k** which affects the density of the ear has probably been changed.

The differences between types (1) to (3) and the corresponding types (4) to (6) are due to the extra chromosomes of the former, which carry a factor **K′** having an effect similar in nature to that of **K** or $\mathbf{K_S}$. The formulae of the types in question may be written:

persicum = (**kk**) + some other change	$\left.\begin{array}{l}\textit{turgidum}\\\textit{durum}\end{array}\right\}$ = (**KK**)	*dicoccum* = ($\mathbf{K_S K_S}$)
vulgare = (**kk**) **K′K′**	speltoid = (**KK**) **K′K′**	*Spelta* = ($\mathbf{K_S K_S}$) **K′K′**

where factors enclosed in brackets are carried by chromosomes that pair in the species hybrids, and K' is carried by the extra chromosomes of the *vulgare* group.

That k, K and K_s are related as multiple allelomorphs is supported by the fact that all have a similar linkage value with the factor for awns. The identity of K in speltoid with K in *turgidum* has been shown in a previous paper. The identity of K_s in *dicoccum* with K_s in *Spelta* is supported by a cross between *turgidum* and *Spelta* studied by the writer, and by various intergroup crosses described by other workers. That the extra chromosomes carry K' is suggested by a morphological comparison between appropriate types.

The theory put forward is not regarded as conclusively proved, but undoubtedly gives a simple explanation of a large number of complicated facts. Though supported by a morphological comparison between the types in question it shows, if true, that in two groups of forms differing genetically in some fundamental way (here the difference is the extra chromosomes of the *vulgare* group) the same series of factor changes (here from k to K and K_s) may cause morphological changes that are not quite parallel in the two groups, and produce types that do not appear, phenotypically, to be related by the same factors.

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PLATE I

a   *b*   *c*   *d*

e   *f*   *g*
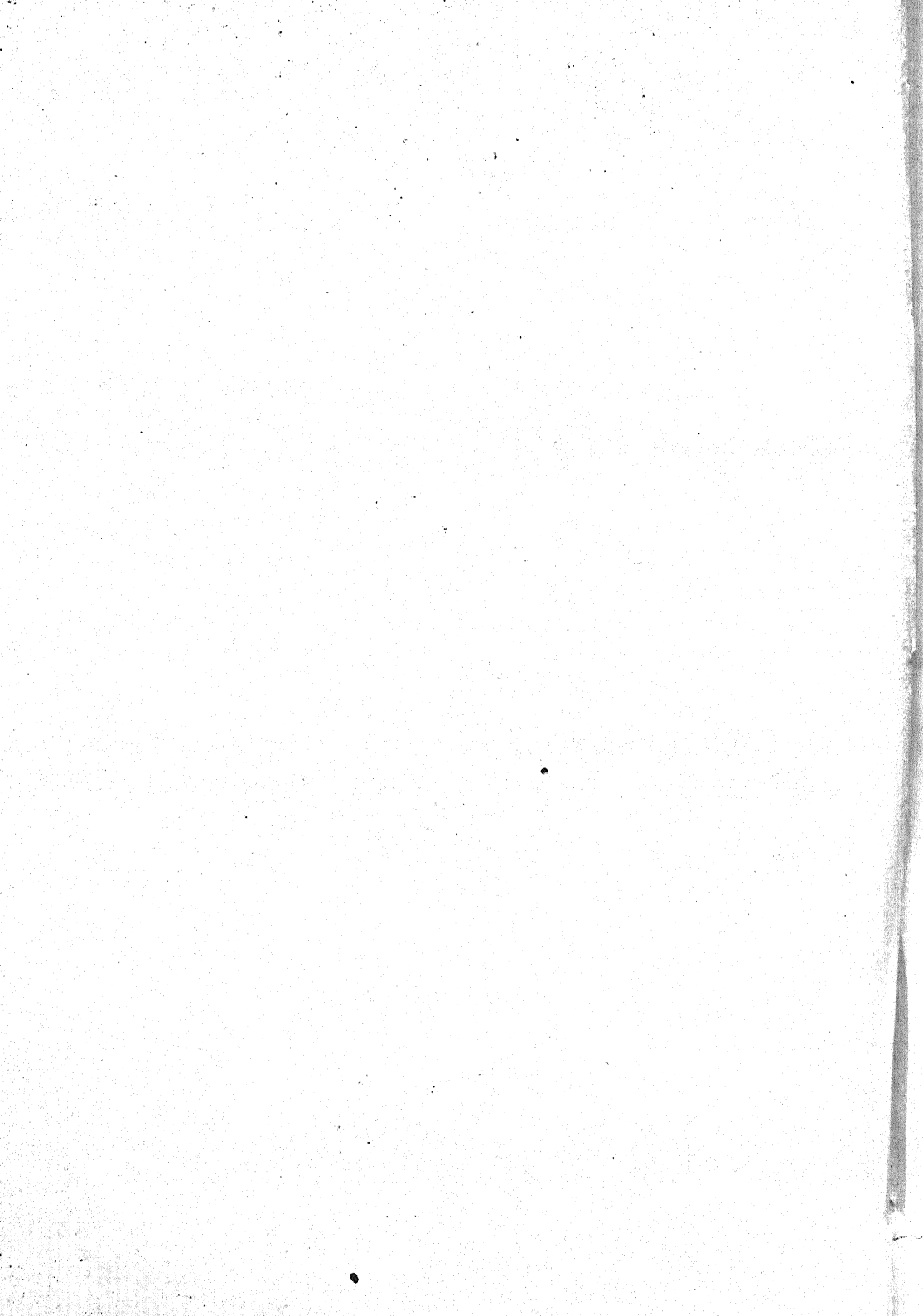
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EXPLANATION OF PLATE I.

Fig. 1. Ears of (*a*) *T. persicum* var. "Persian Black," (*b*) round-glumed *turgidum* extracted from the cross "Swedish Iron" × "Rivet," (*c*) *T. turgidum* var. "Rivet," (*d*) *T. dicoccum* var. "Ajar," (*e*) *T. vulgare* var. "Swedish Iron," (*f*) speltoid extracted from the cross "Swedish Iron" × "Rivet," (*g*) *T. Spelta* var. "White Spelt." All × $\frac{7}{15}$.

INHERITANCE OF STRUCTURAL TYPES IN THE DORSOSACRUM OF DOMESTIC POULTRY.

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(With Four Text-figures.)

CONTENTS.

I. INTRODUCTION.

THE pelvis in different races of domestic poultry exhibits considerable variability, extending over a whole series of characters. Indications of the hereditary nature of many of these exist, and there is no doubt about the genotype of the pelvis of the domestic fowl being very complicated. The structure of the pelvis as a whole constitutes a morphological expression, the phenocomplex of the whole genotype of a given individual; the different characters of the pelvis being correlated with one another both genetically and physiologically, and the pelvic structure of one kind or another being the result of a whole series of genes.

In the present paper, special attention has been paid to the structure of the anterior vertebrae of the pelvis, *i.e.* of its dorsosacral part. This region of the spine shows sharp and distinct differences of structure, and is therefore better suited for genetical analysis. However, here also, in spite of a comparatively clear morphological picture of the types, we find a number of modifications which are perhaps of a fluctuating character. Nevertheless, a great many crosses among poultry confirm the hypothesis we have drawn up of the genetical factors for a given character, and allow us to regard the genetic scheme presented further on as corresponding fairly closely with reality.

The genetical analysis of anatomical characters presents a number of difficulties. Chief among them, in our researches, was the impossibility

of knowing the phenotype of the individual before its death. Only after the death of the parents did the series of their dissected offspring acquire positive value. In this way many crosses could only be utilised post factum, or had to be made according to the supposed structure of the individual. Hence suppositions as to the structure of the pelvis, made on the basis of the genetical working hypothesis, on realisation became of importance. Many chicks from still living poultry could not be completely utilised as the structure of the parental pelvis was entirely unknown.

The materials used for these researches were the adult poultry and chickens of the Anikovo Genetical Station near Moscow. The total number of individuals dissected was about 4000. The crosses principally employed were those of Orloff and Pavloff poultry with Minorcas, Faverolles, Indian Game, etc. Adult fowls and chickens of later embryonic stages were dissected.

The first part of these researches, carried out under the direction of Professor A. S. Serebrovsky, was printed in the *Memoirs of the Anikovo Genetical Station of Moscow*[1] under the title "The genetical analysis of the structure of the pelvis in the domestic fowl." The second part "Types of structure of the sacral region of the pelvis in the domestic fowl and their inheritance," also printed in Russian[2], presented a continuation and a further development of the first part. It introduced some slight alterations in the details of the genetical scheme already put forward, resulting from the study of a larger quantity of material as well as from the completion of various pedigrees through the death of the parents. The present article covers the ground of the earlier ones with some additions.

II. Description of the Pelvic Types.

Normally, domestic poultry have 14 ribless cervical vertebrae and seven pairs of ribs, belonging to V. 15–21. This last is the first vertebra belonging to the synsacrum. According to Gadow(1) V. 21 is the beginning of the dorsosacral region of the pelvis.

The pelvis of a "normal" fowl has *four dorsosacral* vertebrae (21–24), characterised by well-developed transverse outgrowths (processus transversi). The proc. transv. ventrales of V. 24 are especially well developed. Seen from below they appear as broad flat plates, extending from the

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[1] *Genetics of domestic fowl.* Edit. Novaia Derevnia, 1926 (in Russian).

[2] *Russian Journal of Experimental Biology* (edited by Professor N. K. Koltzoff), Série A, II. Nos. 2–3, 1926.

sides of the vertebrae to the os innominatum (Fig. 1, *p*). Sometimes
V. 23 also bears such outgrowths (Fig. 4 *a*), but for the most part they
are somewhat less developed. In the majority of poultry with four dorso-
sacral vertebrae V. 23 resembles V. 22 in structure. It bears com-
paratively thin and concrescent proc. transv. ventrales and dorsales,
which seem to bend inwards towards the back if the pelvis be looked at
from the ventral side (Fig. 1). These variations in the degree of develop-
ment of the proc. transv. of V. 23 exist in many families (series) of
poultry and will be spoken of later on (Section v).
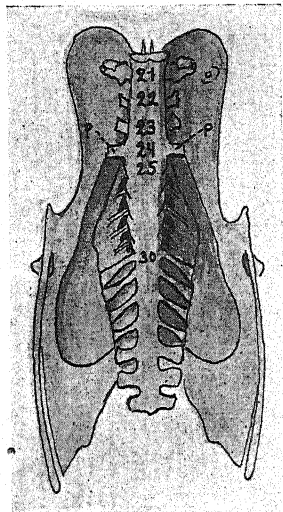
Fig. 1.

With V. 25 in a normal pelvis the lumbosacral region starts, with
vertebrae of an entirely different structure. There are no proc. transv.
ventrales and the proc. transv. dorsales are thin, rising sharply towards
the back and directed somewhat backwards (Fig. 1). After the lumbo-
sacralia, the proc. transv. ventrales begin for the most part from V. 30,
but this character presents a certain variation, and is not taken into
account in this article.

Thus the pelvis of a normal fowl is characterised by *four vertebrae*
of dorsosacral structure, there being a very sharp difference between V. 24
and V. 25. Nearly 75 per cent. of the entire material at my disposal
belonged to this type. The changes in structure of the pelvis, when
compared with the generally accepted anatomical norms, are as follows.

A pelvis with *five dorsosacral* vertebrae is rather frequent in some races of poultry (1000 out of 4000 in our material). V. 25 acquires the same broad proc. transv. ventrales as those of V. 24, and if one looks at the pelvis from the ventral side, the eye is struck at once by *two pairs* of broad flat proc. transv. (Fig. 2, p_1, p_2) going from the spine to the bones of the pelvis, and in front of them three more vertebrae (21–23) with feeble proc. transv. The lumbosacral vertebrae begin with V. 26,
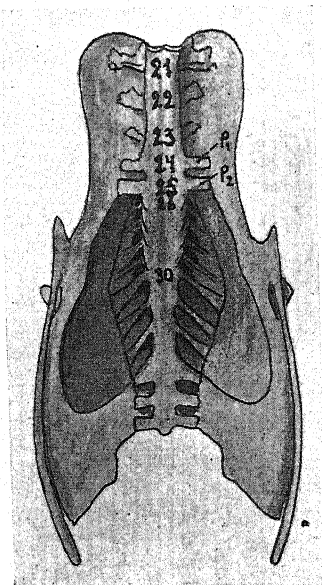
Fig. 2.

and this backward homoeosis of the dorsosacral region often extends to those parts of the synsacrum which are situated behind (the first pair of proc. transv. ventrales after the lumbosacralia begins at V. 30). Frequently V. 22 bears an eighth pair of thin ribs.

This type of pelvis differs sharply from the one described above (having four dorsosacralia), and from its general correlation of the pelvic bones with the synsacrum, creates the impression of a removal backwards along the spine of the different anatomical regions of the synsacrum and of the pelvic bones.

For this reason I have already called it(2) "a type of a backward removal of the pelvic bones" (plus-type, Fig. 2).

The dissection of Bantam chickens and adults has revealed a third

type of pelvic structure, differing sharply from the two types already described in the number of the dorsosacral vertebrae. For in many Bantams the pelvis has only *three dorsosacral* vertebrae (21–23). V. 24 has no proc. transv. ventrales, but from its structure belongs to the lumbosacralia (see Fig. 3). Broad flat proc. transv. (*p*) are also developed here on the last dorsosacral vertebra, viz. V. 23 (not V. 24). Minute dissections made in 1919–20 by A. S. Serebrovsky (at the Toula Genetical Station), and later on by myself, showed only 13 ribless cervical vertebrae in many Bantams. C.V. 14 often had a short rib, and the vertebrae
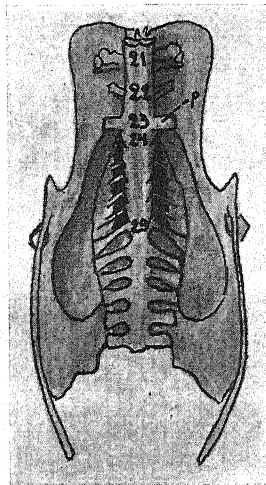
Fig. 3.

14 to 20–21 had seven or eight pairs of ribs. This diminution in the number of the ribless cervical vertebrae considerably obscured the morphological and genetical relations between the Bantam pelvis and that of other types, making their comparison by no means easy. Looking at such a pelvis from the ventral side (Fig. 3), one sees at once, on comparing with a normal pelvis (Fig. 1), and particularly with the type possessing 5 ds. vertebrae (Fig. 2), that the anatomical regions of the synsacrum, and to some extent the bones of the pelvis, seem to have been moved forward. This displacement I named (in the first paper) a "forward removal."

Therefore, according to the number of dorsosacral vertebrae, three principal types can be established in the structure of the pelvis (see Table I).

TABLE I.

Types of pelvis	Bantam type: Conventional designation 3 ds.	Normal type: Conventional designation 4 ds.	Lengthened type: Conventional designation 5 ds.
Number of dorsosacral vert.	3	4	5
Dorsosacral vert. (as counted from the cranium)	21–23	21–24	21–25
Broad proc. transv. (p) (see Figs. 1–3)	Constantly on the 23rd vert.	On the 24th or on the 23rd and 24th vert.	Constantly on the 24th and 25th vert.

To elucidate the inheritance and genetical nature of these three types is the object of the present paper. But before attempting to do so it is necessary to make a brief study of the variability within the limits of each type. A general examination of my material showed a somewhat considerable variation in the normal 4-ds. type, manifested by a greater or lesser development of the proc. transv. on V. 23. In my first paper, the 4-ds. type of structure was even divided into two types, viz.

(1) The so-called "typical structure" (N 4 ds., see Section v) where the proc. transv. of V. 23 are like those of V. 22, and much less developed than on V. 24 (Fig. 1 or 4 c).

(2) The so-called "incomplete removal forward" (M 4 ds., Section v) where the proc. transv. of V. 23 are of the same size as those of V. 24 (Fig. 4 a).

These two types of structure are united by a chain of transitions which, conventionally, can be thus expressed:

proc. transv. of V. 23 = of V. 24...23 $\leq$ 24...23 < 24...|...23 $\ll$ 24...22 = 23 $\ll$ 24..."the typical structure."

The structures placed to the left of the vertical line, I considered as the "removal forwards" (Fig. 4 a, b), that to the extreme right as the typical structure (Figs. 1 and 4 c, d). More extensive material and a more minute examination, however, have now led me to the conclusion that the common fundamental character is the presence of *four dorsosacral* vertebrae, the variability in size of the proc. transv. of V. 23 (causing the differences described above) being a character of secondary importance, with evidently some phenotypical variation. (Some indications of this hereditary character will be mentioned later in Sections III and v.) Hence in the subsequent course of my work cases of "incomplete forward removal" were united with those of typical structure, owing to the presence of four dorsosacral vertebrae, into one and the same 4-ds. type, as being genetically and morphologically a distinct one.

The identity of these two structures is also manifest by the fact that the proc. transv. of V. 23 and V. 24 are not very apparent, and present but slight differences in embryos and newly-hatched chickens. Only through later ossification and concrescence of the vertebrae do the relative sizes of the proc. transv. of these vertebrae become quite evident.

Besides those in V. 23 there are other variations in this type of pelvis found in V. 25. This vertebra, the first of the lumbosacralia, has usually no proc. transv. ventrales in this type, thus differing distinctly from the
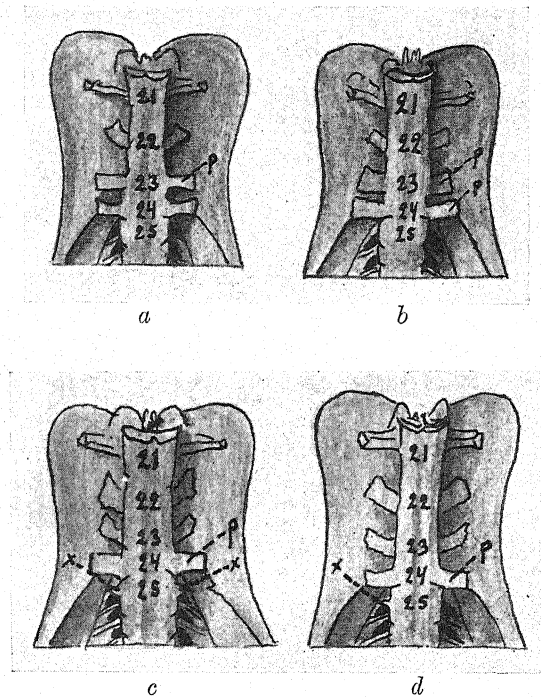
Fig. 4.

preceding vertebra, the last of the dorsosacral ones (24). However, in a few cases (about 1 per cent.) small conical protuberances are noticeable on V. 25—the rudiments of the proc. transv. ventrales, which do not reach the os innominatum, and are free at the end. Sometimes these protuberances only exist on one side (see x on Fig. 4 c, d). The feeble development of these rudimentary formations compels us to class such structures among the 4-ds. type.

The type with 5 dorsosacral vertebrae (5 ds.) varies very little, as

V. 24 and V. 25 are nearly always very much alike, both in structure and in the degree of development of the proc. transv. ventrales. Owing to the presence of *two pairs* of broad proc. transv. (*p*), this type seems, at first sight, to be very like the variation of 4 ds., where the proc. transv. of V. 23 are as strongly developed as those of V. 24 (comp. Figs. 2 and 4 *a*). But they belong to different vertebrae (23–24 and 24–25). What is of importance, is that in spite of the morphological resemblance in the structure of the vertebra possessing *p*-outgrowths (see Figs. 2 and 4 *a*), and although the position of V. 24 is analogous to that of V. 23 in the 4-ds. type (viz. in front of the last vertebra of the dorsosacral structure), in this (24th) vertebra (in the 5-ds. type) the degree of development of the proc. transv. does not show the slightest variation, and also all the individuals of the 5-ds. type have two, almost equal, pairs of broad proc. transv. ventrales, belonging to V. 24 and V. 25 (about 1000 individuals). This absence of variation in the proc. transv. of V. 24 (for the type given) finds its explanation in the genetical interpretation of the character investigated (see end of Section v, and Section viii). On the whole, the type with 5 ds. vertebrae is very constant and well defined.

Variation in the 3-ds. type is likewise small. V. 22, which precedes V. 23 with broad proc. transv. (*p*), never bears such proc. transv. but always ones of a distinct type—narrow and situated deeply inwards (Fig. 3). The only rare deviations found were pointed excrescences on V. 24—the rudiments of proc. transv. ventrales of the dorsosacral type. These outgrowths do not acquire the shape of the proc. transv., and there is therefore no difficulty in referring this structure to the 3-ds. type. These changes are very like those already described for V. 25 in the normal pelvis (Fig. 4 *c*, *d*).

In concluding this descriptive part I may state briefly the correlations existing between the types of pelvis described and the number of the ribs. The normal number of ribs in poultry is seven pairs but not infrequently there is found an extra pair. This eighth pair can have two modes of origin, appearing either anteriorly on C.V. 14 or posteriorly on V. 22. The first mode occurs in Bantams, leading to the formation of 13 ribless vertebrae in the cervical section. In other races the extra ribs are nearly always found posteriorly on V. 22 and then show a close correlation with the 5-ds. pelvic type. This connection can be illustrated as follows:

(1) In 386 chickens, possessing 14 cervical vertebrae and eight pairs of ribs (the extra one on V. 22), the distribution of the pelvic types was as shown in Table II.

TABLE II.

	Types of pelvis	Number of chickens
14 cervical vertebrae and 8 pairs of ribs	4 ds.	106
	5 ds.	280

(2) In seven-ribbed chickens the proportions were as in Table III.

TABLE III.

	Types of pelvis	Number of chickens
14 cervical vertebrae and 7 pairs of ribs	4 ds.	296
	5 ds.	102

A correlation is very evident. The elongation of the dorsosacral region from 4 to 5 vertebrae is frequently accompanied by an extra pair of ribs. 106 chickens of the 4-ds. type (see Table II) all had V. 23 resembling V. 22 (Fig. 1) and there was not a single modified chicken that had a V. 23 like V. 24 (Fig. 4 a). It is difficult to decide the nature of such correlation and the question is evidently one for further research.

III. Illustrations of the Hereditary Nature of the Characters Studied.

The hereditary nature of the types described is apparent even after a most superficial examination of the data derived from crosses. I give the following examples:

(1) 20 crosses of the type 4 ds. × 4 ds. have given 324 chickens, viz. 252 of the 4-ds. type, and 72[1] of the 5-ds. type (= 77·8 : 22·2 %).

(2) 20 crosses of the type 4 ds. × 5 ds. gave 316 chickens, viz. 220 of the 4-ds. type and 96 of the 5-ds. type (69·6 : 30·4 %).

(3) 3 crosses of the type 3 ds. × 4 ds. gave:

$$24 \text{ 3 ds.} : 25 \text{ 4 ds.} : 0 \text{ 5 ds.}$$

(4) 3 crosses of the type 4 ds. × 5 ds. gave:

$$0 \text{ 3 ds.} : 16 \text{ 4 ds.} : 15 \text{ 5 ds.}$$

There are a few crosses of the type 4 ds. × 4 ds. which gave only chicks of the 4-ds. type.

The data in the column to the right in Table V (p. 43) may serve as examples of the numerical relations for the different pairs of parents. The existence of a genetical segregation in the offspring is distinctly manifested by the following data. Among the material there are fowls—sisters and brothers—which, when respectively crossed with the same

[1] Further on in the formulae the 4-ds. type will be written first, the 5-ds. type second; and in the crossings with the 3-ds. type, this pelvic type will be placed first.

cock (or with the same hen) give a progeny differing in respect of the numerical proportions of the pelvic types. The differences are marked, and there can be little question of their showing that the sisters (or brothers) we are comparing belong to different genotypes. Thus two sister hens 3837 and 3838 (daughters of Bantam-Pavloff ♂ F_1 2328 4 ds. and Paduan ♀ 1202 M 4 ds.) were crossed with one and the same cock 4949 (Bantam hybrid; pelvic structure unknown) and produced different series of chickens:

		M 4 ds.	N 4 ds.	5 ds.		M 4 ds.	N 4 ds.	5 ds.
(a)	♂ 4949.♀ 3837	4	: 17	: 2	or	17·4	: 73·9	: 8·7 %
(b)	♂ 4949.♀ 3838	14	: 15	: 3	or	43·7	: 46·9	: 9·4 %

The real deviation of these series from each other is more than three times as great as the sum of the probable errors of the classes, thus showing with certainty that the hens 3837 and 3838 belong to different genotypes. The comparison between the two series led one to suppose, in the hen 3838, the presence of a M 4-ds. type (see Section v, Fig. 4 *a*) and this was confirmed by a *post mortem* dissection of this hen.

As a second example we may take the two brothers, F_1 Indian Game-Pavloff, which when mated with the same two hens (their sisters) gave entirely different series:

	M 4 ds.	N 4 ds.	5 ds.
(a) ♂ 2123 with two of his sisters gave	14·5	: 71·0	: 14·5 %
(b) ♂ 2124 with the same sisters gave	35·7	: 57·7	: 7·5 %

♂ 2123 had the normal 4-ds. pelvic type. It was supposed that the cock 2124 had the M 4-ds. type (Section v) and so it turned out after his death.

Such cases unquestionably point to the hereditary character of the types of pelvic structure described. If a fluctuation does exist, it does not markedly overshadow the genetical tendencies, and we may now discuss the possibility of framing a genetical scheme of inheritance.

Structurally the 3-ds. type is more sharply marked off from the two other types, being found only in Bantams and their hybrids. Its genetical relation with the others will be examined further on in Section vi. We may now trace the genetical relations of the 4-ds. and 5-ds. types.

IV. Inheritance of Pelvic Types with Four and Five Dorsosacral Vertebrae.

A general examination of all the material obtained in dissecting chickens from parents of known pelvic structure brought out the following points:

(1) The very great frequency of chickens of the 4-ds. type (in all about 3000 out of 4000).

(2) Crosses of the 4-ds. × 4-ds. type gave either all the chickens of the 4-ds. type, or else up to 40–50 per cent. (in separate families) of chickens of the 5-ds. type.

(3) Crosses of the 5-ds. × 5-ds. type (unhappily but few in number) gave, for the most part, a small number of individuals of the 4-ds. types, the rest being like the parents.

(4) Crosses of the nature 4 ds. × 5 ds. nearly always gave both types, sometimes in equal proportion, or else not less than $\frac{1}{4}$ of the 5-ds. type.

On comparing points (2) and (3) one can at once see that the genetical relations of the 4-ds. and 5-ds. types are rather complex. Two apparently contradictory results, viz. (a) 4 ds. × 4 ds. gives 5 ds. and (b) 5 ds. × 5 ds. gives 4 ds., do not admit of a simple Mendelian relation between these two types. The high proportion of the 4-ds. type in the general population suggests that it is not recessive. I pointed out the dominant nature of this type in my first paper, where the regular appearance of chickens of the 5-ds. type from individuals of the 4-ds. type was shown.

The study of the genealogy and of the entire progeny of 4-ds. type individuals has revealed in some of them a difference of behaviour on crossing with the same cock, or the same hen. It appeared possible to isolate two groups of individuals with a pelvic structure of the 4-ds. type.

The majority of hens (Group I) in crosses with the 5-ds. type transmit the 4-ds. type in marked degree, but a few (Group II), even in crosses with the 4-ds. type, show in their progeny a definite preponderance of the 5-ds. type. For example:

1. Orloff hen 709 (4 ds.) with cock Orl. 708 (5 ds.) has given 11 4 ds. : 4 5 ds. (Group I).
   Orloff hen 710 (4 ds.) with the same cock (708) has given 11 4 ds. : 22 5 ds. (Group II).
2. Hen 3297 (4 ds.) with cock 2123 (supp. 4 ds.) has given 11 4 ds : 0 5 ds. (Group I).
   Hen 1985 (4 ds.) with the same cock (2123) has given 36 4 ds. : 8 5 ds. (Group II ?).
3. Plymouth Rock hen 669 (4 ds.) with English Game cock 605 (4 ds.) has given 4 4 ds. : 3 5 ds. (Group I).
   Hybrid English Game hen 1227 (4 ds.) with the same cock (605, her father) has given 1 4 ds. : 6 5 ds. (Group II).
4. The three 4-ds. hens 15, 3838, 2454 in crosses with cocks of the 5-ds. type gave only chickens of the 4-ds. type (homozygotes 4 ds.?).

These examples show that the 4-ds. type evidently possesses several different genotypes. Individuals of the 4-ds. type derived from 5 ds. × 5 ds. matings are clearly genetically distinct from those which, when crossed with the 5-ds. type (example 4, above), give chickens of the 4-ds. type only. We have also the following crosses:

(a) 4 ds. × 5 ds. (15.57)   gave 6 4 ds. : 0 5 ds.
(b) 4 ds. × 5 ds. (2015.2437) „ 5 „ : 7 „
(c) 5 ds. × 5 ds. (57.2337)   „ 5 „ : 8 „
(d) 5 ds. × 5 ds. (3246.2337) „ 7 „ : 8 „

In (a) the 4-ds. type seems to have been completely dominant over 5 ds. In (b) there is approximately an equal number of each type, and the 4-ds. type of the mother behaves like a heterozygote (?). In (c) and (d) we appear to obtain chickens of the 4-ds. type as extracted recessives from 5-ds. parents. There would seem to be both a dominant and a recessive type of 4-ds. pelvis.

We may suppose the existence of two different factors not allelomorphic to one another, viz. **A**, for the 4-ds. type, and **B**, for the 5-ds. type of pelvic structure. From the sample crosses given above (*e.g.* 4 ds. × 4 ds. giving 4 ds. and 5 ds.) we may further suppose that **A** is epistatic to **B**, so that **aB** individuals will have the 5-ds. structure, and **AB** (and **Ab**) individuals the 4-ds. structure.

In an earlier paper I suggested that the genotype **aabb** is of the 4-ds. type, regarding it as a normal recessive. The appearance of 4-ds. individuals in the cross 5 ds. × 5 ds. (**aB** × **aB**) is evidently possible only if both parents are heterozygous for **B**, for these "extracted" individuals must be **bb**. Also they cannot contain **A**, since this, on our hypothesis, is epistatic to **B**. Hence the recessive 4-ds. type must be **aabb**. Possibly there may be another factor, hypostatic to **A** and **B**, which in their absence stimulates the 4-ds. phenotype, though it is simpler to suppose that **a**, in the absence of **B**, brings about the 4-ds. type of pelvis.

The following individuals may serve as examples of **aabb** genotypes:

(1) Orloff ♀ 710 (4 ds.) with ♂ Orl. 708 (5 ds.) gave 11 4 ds. : 22[1] 5 ds.

(2) Orloff ♂ 6987 (4 ds.) *ex* 5 ds. × 5 ds. His genetical behaviour when mated with 5-ds. ♀♀ suggests that he is a recessive 4-ds. bird; for with ♀ 6797 (suppos. 5 ds.) he gave 3 4 ds. : 3 5 ds.; with ♀ 3317 (suppos. 5 ds.) he gave 3 4 ds. : 2 5 ds. Unfortunately the numbers are very small as the cock died early.

Hence for these two types of pelvis we suggest the following genetical scheme:

A, a factor stimulating a pelvic structure with 4 ds. vertebrae, *i.e.* inhibiting the action of **B**.

a, a recessive gene for the pelvic structure with 4 ds. vertebrae.

B, a factor stimulating a pelvic structure with 5 ds. vertebrae.

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[1] In this case there is evidently a lack of **aabb** (4-ds.) chickens, possibly owing to their lower viability. However, the data are not yet sufficient to test this supposition.
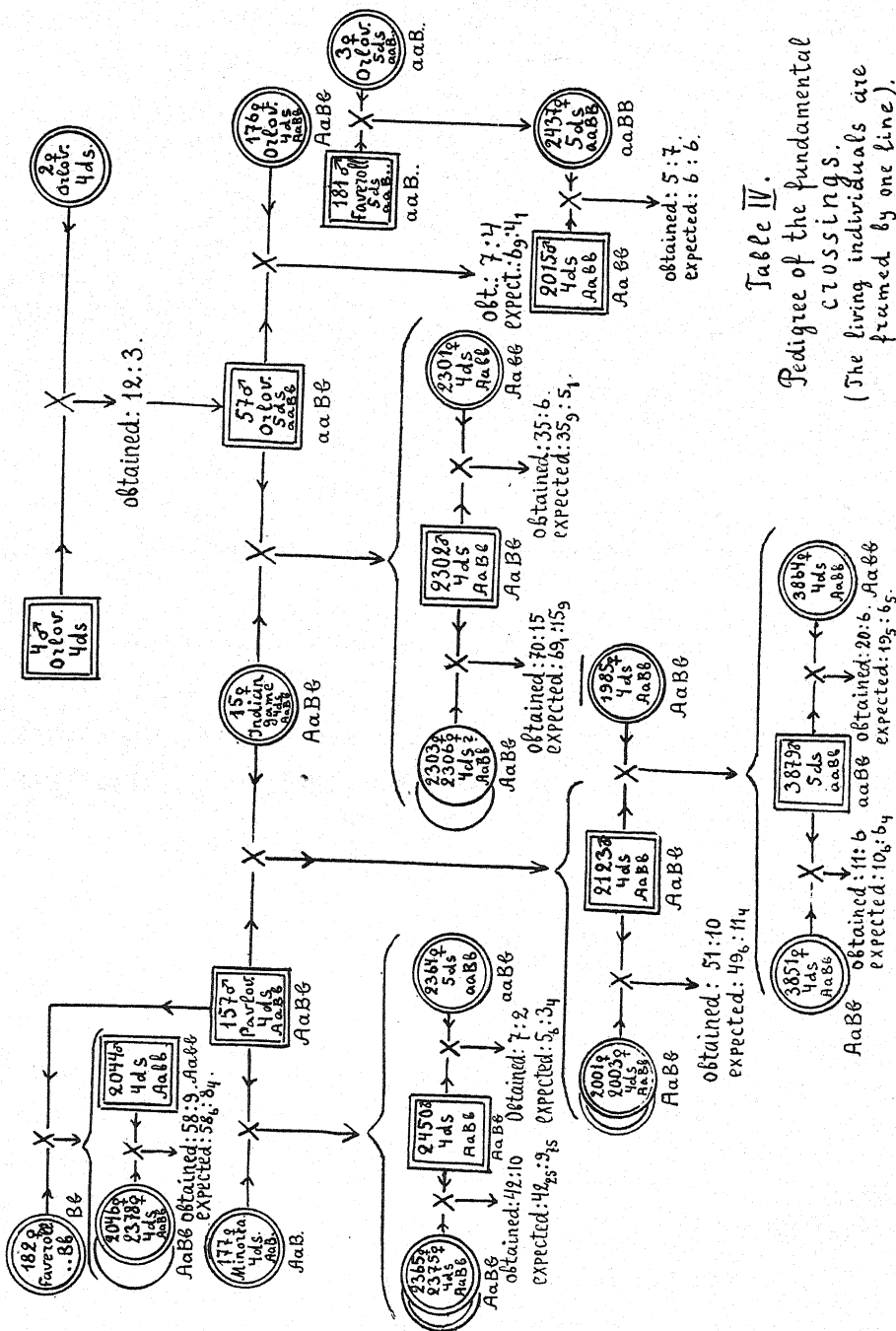
Table IV.

Pedigree of the fundamental crossings.

(The living individuals are framed by one line).

b, its recessive allelomorph (participates perhaps in the modification of the 4-ds. type; see Section v).

A is almost completely epistatic over **B**.

a is hypostatic to **B**.

Individuals **AaBb, Aabb** = 4-ds. type[1] (including the modifications); **aaBB, aaBb** = 5-ds. type; **aabb** = 4-ds. recessive type.

This genetic scheme fits closely the actual data derived from the crosses. The possibility of a successful forecast of the pelvic structure and a very close coincidence of the numbers presupposed by the scheme with those obtained (see pedigree, Table IV), show that the scheme accords well with the facts.

Examples of genetical segregation and of inheritance of the 4-ds. and 5-ds. types in different families and pedigrees are given in Tables IV and V. Table V contains the greater part of the data derived from crosses of parents whose pelvic structure is known. Of crosses where at present the structure of only one parent is known, we have a much greater number, but I shall not give them as one cannot employ them with absolute certainty. Also there are many dissections of chickens which cannot be utilised since both parents are still alive. The many *post mortem* confirmations of forecasts of structure made on our hypothesis allow of our pronouncing with definite probability on the pelvic structure of a living individual from the progeny results on crossing; *e.g.* the birds 2001, 2003, 2044, 2046, 2123, 2302 in Table IV were, when still alive, placed in the 4-ds. type, and 2437 in the 5-ds. type; and this was confirmed after death. The individuals 2303, 2306 are still alive and are considered as belonging to the 4-ds. type (see pedigree).

The following cases may serve as examples of the method of assigning formulae:

A 4-ds. structure was presupposed for ♂ 2302 from the numerous crossing results taken altogether. With 4-ds. individuals there were rather numerous crossings on the 3 : 1 scheme, and some large series which gave a small number of 5-ds. type, very close to a 7 : 1 ratio.

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[1] In my first researches referred to above I isolated the extreme modification and the development of the proc. transv. of V. 23 in the 4-ds. type, into a separate type (the so-called "incomplete forward removal," Fig. 4 *a*) with the genetical formula **Abb**. This formula has now been confirmed for such a variation (see Section v), but the structure itself is classed with my earlier "typical" structure (Fig. 1) to form one common 4-ds. type (the number of the dorsosacral vertebrae, being the fundamental character). Hence **A** must be regarded as the gene for the 4-ds. type, and not only for the "removal forward" (**Ab** is not only a "removal forward," but the 4-ds. type in general). For the rest, the genetic scheme remains the same (concerning Bantams, see Section vi).

TABLE V.

Examples of the inheritance of 4-ds. and 5-ds. types.

Crossing	Expected according to the scheme	Genetic formulae	Obtained 4 ds.	Obtained 5 ds.	Expected 4 ds.	Expected 5 ds.	Remarks Isolated pairs
4 ds. × 4 ds.	1 : 1	**aabb × AaBB** (4 pairs)	17	17	17	17	6 : 6; 4 : 6
„	All 4 ds.	Homozygotes **AA** (6 pairs)	64	—	64	—	—
„	3 : 1*	**AaBb × AaBB** (6 pairs)	64	23	65·25	21·75	11 : 4; 8 : 3; 21 : 6
„	7 : 1	**AaBb × Aabb** (6 pairs)	149	22	149·6	21·4	34 : 5; 27 : 4
„	13 : 3*	**AaBb × AaBb** (4 pairs)	133	31	133·25	30·75	36 : 8; 35 : 8
4 ds. × 5 ds.	All 4 ds.	Homozygotes **AA** (3 pairs)	16	—	16	—	—
„	1 : 1	**AaBb × aaBB** or **AaBB × aaBb** (4 pairs)	22	19	20·5	20·5	6 : 6; 5 : 7
„	5 : 3	**AaBb × aaBb** (3 pairs)	53	31	52·5	31·5	11 : 6; 25 : 15
„	3 : 1	**Aabb × aaBb** (6 pairs)	77	24	75·75	25·25	19 : 7; 20 : 6
5 ds. × 5 ds.	1 : 3	**aaBb × aaBb** (3 pairs)	6	16	5·5	16·5	2 : 5; 3 : 7
„	All 5 ds.	Homozygotes **BB**	There are no crossings				

* The examples for the schemes 3 : 1 and 13 : 3 are approximate, as with a small numerical quantity and incomplete pedigrees, one cannot be sure which of these schemes we have for a given family. (The calculation of the probable error for separate families often does not give here any definite indications.)

Hence ♂ 2302 cannot be homozygous for **A** since in that case he would give only chickens of the 4-ds. type. Nevertheless he must carry **A** since his behaviour is not that of the recessive **aabb**. Again, one must suppose that he also carries **B** for the following reason. In some of his crosses with 4-ds. ♀♀ carrying **B** the number of 5-ds. offspring chickens nearly approaches one-quarter. On our hypothesis such a result is given by the following three types of cross, viz.

AaBb × AaBB
AaBb × aabb
AaBB × AaBB.

All individuals here, except the recessive **aabb**, have the factor **B**. Now suppose for a moment that ♂ 2302 is a 5-ds. bird. The cross 4 ds. × 5 ds. only gives a 3 : 1 ratio when it is of the nature **Aabb × aaBb**, *i.e.* where the individual of the 4-ds. type does not carry **B**. But the females of the 4-ds. type crossed with ♂ 2302 all carried **B**. Therefore the appearance of a 3 : 1 ratio when they were crossed with ♂ 2302 shows

that he must contain **B**, and consequently belong to the 4-ds. type. And the existence of modified 4-ds. individuals in his offspring points to his being heterozygous for **B**. On these grounds he was supposed in my first paper to be constitutionally **AaBb**, a supposition that has lately been confirmed *post mortem*.

Again, ♀ 2437 came of two 5-ds. parents (see pedigree) and when crossed with ♂ 2015 (4 ds.) gave a large number of 5-ds. chickens. Consequently she could not have been **aabb**, and hence presumably belonged to the 5-ds. type, a presumption that was confirmed after her death. Further the approximate equality of the 4-ds. and 5-ds. types from the cross with ♂ 2015 (**Aabb**) suggests that she was **BB** rather than **Bb** (with **Bb** 3 : 1 was the expected ratio).

The calculation of a formula for an individual whose pelvic structure is known is much simplified by crossing with many different hens (or cocks).

For example, ♂ 3879 (see pedigree on Table IV) of the 5-ds. type came from ♂ 2123 (4 ds. **AaBb**) and ♀ 1985 (4 ds. **AaBb**), who gave between them 36 4-ds. and 8 5-ds. chickens—a close approach to the expected 13 : 3 ratio.

♂ 3879 with certain of his sisters gave the following results:

(1) With ♀ 3851 (4 ds.) he gave 11 4 ds. : 6 5 ds.
(2) With ♀ 3864 (4 ds.) he gave 20 4 ds. : 6 5 ds.
(3) With ♀ 3877 (supp. 4 ds.) he gave 16 4 ds. : 9 5 ds.

Families (1) and (3) are much alike and suggest the type of mating **AaBb** × **aaBb** giving a 5 : 3 ratio.

Family (2) suggests a 3 : 1 ratio from the mating **Aabb** × **aaBb**. All three results agree with the supposition that ♂ 3879's formula is **aaBb**.

V. Hereditary Modifications of the Pelvis with Four Dorsosacral Vertebrae.

The modifications of the 4-ds. type, as regards the development of the proc. transv. ventrales of V. 23 (Fig. 4 *a*, *b*), have been described in Section II. A series of transitions, apparently of a fluctuative character, exists between the structures represented in Figs. 1 and 4 *a*. One of them is shown in Fig. 4 *b*.

Nevertheless, the pelvis with *strongly* developed proc. transv. on V. 23 (p V. 23 = p V. 24, Fig. 4 *a*) often differs genetically from the normal structure (Fig. 1). As I have already pointed out, this structure was previously regarded as a separate type, and in my first paper[2] definite data were given as to its hereditary nature, and a formula

was devised for it in terms of **A** and **b**. When subsequent work cleared up the genetical nature of the differences between the various pelvic types it was classed with those of normal structure, both having 4-ds. vertebrae. In the examples discussed above I did not separate this subtype, as these examples were intended to illustrate the inheritance of the *number* of vertebrae in the dorsosacral structure.

We may now discuss the genetical nature of this modification in terms of the scheme already put forward.

On this scheme the 4-ds. type can have seven genotypes, viz.

     (1) **AABB**;   (2) **AABb**;   (3) **AAbb**;
     (4) **AaBB**;   (5) **AaBb**;   (6) **Aabb**;
     (7) **aabb**;

and we suppose that of these (3) and (6) show the modified structure in most cases while the rest show a normal one. There are many examples in our material which make this hypothesis probable. In agreement with it is the fact that in crosses between two modified 4-ds. individuals (**Ab**), 5-ds. chickens are very rarely seen. But the modified structures show considerable phenotypical variation, especially in the transitional cases, and are difficult to classify. Only individuals of the structure shown in Fig. 4 *a, b* were classed as those of modified structure, the other forms, presenting transitions towards the normal type, being classed with the latter (Fig. 1).

As examples illustrating the hereditary nature of this modification of the 4-ds. type, we may take the following cases (N 4 ds. being the normal, M 4 ds. the modified structure):

    (1) N 4 ds. ♂ 2884 × M ♀ 1202 gave 5 M : 7 N (all 4 ds.).

    (2) M ♂ 2124 × N ♀ 2001 gave 5 M : 6 N (all 4 ds.).

    (3) N ♂ 2302 × N ♀ 2301 gave 3 M : 11 N and 6 5 ds.

The differences in the results of (2) and (3) illustrate the different genetical behaviour of M ♂ 2124 and N ♂ 2302 when mated with the same hen.

The pedigrees in Table IV illustrate the following cases of matings of the type N **AaBb** × N **AaBb** where the expected ratio is 3 M : 10 N : 3 5 ds.:

	M 4 ds.		N 4 ds.		5 ds.
(a) 2450♂.2365♀ gave	4	:	17	:	4
expected	4·7	:	15·6	:	4·7
(b) 2450♂.2375♀ gave	6	:	15	:	6
expected	5	:	17	:	5
(c) 2302♂.2303♀ gave	6	:	31	:	11
expected	9	:	30	:	9
(d) 2302♂.2306♀ gave	8	:	25	:	4
expected	6·9	:	23·2	:	6·9

In all of these the actual results accord closely with expectation.

In several families from **AaBb** × **AaBb** (see Table V, under ratio 13 : 3) 164 chickens were obtained, distributed as follows:

obtained : 15 M : 118 N : 31 (5 ds.), expectation being 30·75 : 102·50 : 30·75.

Here the deficiency of modified individuals and the excess of normal ones together with the close approach to expectation of the numbers for 5 ds. suggest that for the **Ab** combination the modification is not always complete, and that some of the more weakly modified individuals have been classified as normals.

The 1 : 1 ratio expected on hypothesis from the mating **AaBb** (N) × **AAbb** (M) is illustrated by examples 1 and 2 on p. 45.

Crosses of the types **aaBB** (5 ds.) and **Aabb** (4 ds. modif.) should give 4-ds. and 5-ds. types in equal numbers. From ♀ 2437 (cf. pedigree), known to be **aaBB**, we should expect all the 4-ds. chickens to be normal on crossing with a M 4 ds. ♂. Crossed with ♂ 2015 (M 4 ds. **Aabb**) she gave 5 4-ds. and 7 5-ds. chicks, all of the former being unmodified normal ones as expected.

The 5-ds. bird heterozygous for **B** (**aaBb**) when crossed with a modified 4-ds. bird of the constitution **AAbb** should give only 4-ds. chicks, half being modified and half normal. ♂ 3879 (see pedigree, Table IV) known to be **aaBb** was crossed with M 4 ds. ♀ 3838 (**AA**), and all the chickens obtained were of the 4-ds. type, M and N being represented in equal numbers.

We may take it therefore that **B** does actually restrain a strong development of the proc. transv. on V. 23 in the 4-ds. pelvic type.

The **Ab** combination is for the most part, but not always, a modified 4-ds. type, either entirely or feebly.

The combination **AB** is almost always of the normal 4-ds. type. It is possible that the heterozygous or homozygous state of **B** is also of importance for the modification of the 4-ds. type, but the material in hand is insufficient for an analysis of this point. The mutual relations of **A** and **B** explain the absence of variations in V. 24 of the 5-ds. type, as no antagonism between these two factors exists here.

VI. Inheritance of the Pelvic Type with Three Dorsosacral Vertebrae.

Many Bantams have a specific structure of the pelvis with 3 ds. vertebrae (Fig. 3). This structure is often accompanied by changes throughout the spine. Normally domestic fowls have 14 ribless cervical

vertebrae and 7 pairs of ribs, the first pair being borne by V. 15. In all the races of poultry that I have dissected individuals with 7 or 8 pairs of ribs were met with. These changes in the number of vertebrae also exist in Bantams (about 50 per cent. are 8-ribbed), but are much more complicated, owing to the fact that here the eighth pair makes its appearance in a manner different from that usual for the other races of poultry. A normal individual (not a Bantam) with 14 cervical vertebrae and 7 ribs carries these ribs on V. 15–21; in an individual with 8 pairs the extra rib develops on V. 22 and is generally rudimentary. The extra rib appears here *behind* whereas in Bantams I never found an eighth pair on V. 22. Eight-ribbed Bantams had always 13 cervical vertebrae with ribs on V. 14–21, the eighth rib appearing here *in front*.

The study of the pelvis of adult Bantams, and of their chickens in which the anterior limit of the synsacrum region was already quite clear or well marked, and where the concrescence of the synsacral vertebrae was complete or beginning, showed that there was not a single pelvis with 5 ds. vertebrae, though 3 ds. vertebrae were not infrequent. In nearly all cases V. 21 was the first one concrescent with the synsacral one, *i.e.* in numerical order counting from the cranium the same one as in individuals with 14 cervical vertebrae. In the 8-ribbed individuals with 13 cervical vertebrae only the last pair of ribs belongs to a pelvic vertebra, viz. V. 21, and in this respect such a pelvis is similar to the 7-ribbed pelvis of the 3-ds. type shown by individuals with 14 cervical vertebrae, there being one pair of pelvic ribs in each case. The extra pair of ribs always appears in front on C.V. 14. Hence the 3-ds. and 4-ds. types in the Bantams can be compared with the types of other races as regards their different elements (number of pelvic vertebrae, last pelvic ribs, etc.).

Though the Bantam material is not very extensive (about 300 dissections including 114 pure-bred Bantam chickens) it nevertheless allows of some definite conclusions as to the genetical nature of the 3-ds. type.

The pelvic types were distributed as follows in the 114 pure Bantam chickens: 3-ds. type, 50 individuals; 4-ds. type, 64 individuals; 5-ds. type, none.

Genetically Bantams of the 4-ds. type behave like 4-ds. individuals homozygous for **A**. Many of them show the modified form of structure as in Fig. 4 *a*, and one can suppose these to be **bb** birds.

The following cross between Pavloff F_1 hybrids and Bantams may serve as examples of 4 ds. × 4 ds. matings. Three hens (modif. 4-ds. type), crossed with ♂ 2328 (norm. 4 ds.), gave 40 chickens of the 4-ds.

type: 21 modified and 19 normal, *i.e.* the expected equality if the hens were **AAbb** and the cock either **AABb** or **AaBb**.

As an example of a cross between pure-bred Bantams of the 3-ds. type and a 4-ds. individual we may take the case of ♂ 618 (4-ds.) which with 3 ♀♀ gave 24 3 ds. : 25 4 ds.

The 1 : 1 ratio occurred also in the separate families. Unfortunately there were no 3 ds. × 3 ds. crosses with both parents known. From 4 ds. × 4 ds. crosses among Bantams the 3-ds. type did not occur, 4-ds. individuals do not apparently give the 3-ds. type, but I cannot affirm this with perfect certainty as the material studied has not been large.

Of great interest and value is the recently realised cross of the big Orloff fowl with Bantams which has been successful when the Bantam was used as the mother, though the number of chickens studied has been small.

The Orloff cock 708 (5-ds. type) was crossed with a Bantam hen 3223 (3-ds. type) and gave several chickens, 3 of which died and were dissected. One was a 3-ds. type (!) and two were of the 4-ds. type. The appearance of a 3-ds. type in crossing with an Orloff cock, must point to the dominant nature of this type, as it cannot be supposed that a pure-bred Orloff could be heterozygous in a factor for the 3-ds. type, a form of pelvis never found in pure-bred Orloffs or in other large races of poultry. Nor does the possibility of a fluctuation from 5 ds. to 3 ds. seem at all likely.

The dominant nature of the 3-ds. type is also probable from the following data:

Bantam cock 618 (4 ds.) × Bant. hen 3223 (3 ds.) gave 1 3 ds. : 2 4 ds.
Orloff cock 708 (5 ds.) × Bant. hen 3222 (3 ds.) gave 1 3 ds. : 2 4 ds.

We suppose therefore that there is a factor (**C**) for the 3-ds. type epistatic to the factors for the other pelvic types[1] and this is in agreement with the case of the 4 ds. ♂ 618 above which gave practically equal numbers of 3-ds. and 4-ds. chicks from three different hens. Constitutionally the ♂ 618 must have been **cc**, and each of the hens **Cc**.

VII. CONCLUDING REMARKS.

From the data we have given some general conclusions can be drawn as to the distribution of the genotypes of the pelvis in different races of poultry.

In the first place it is evident that pure-bred poultry are scarcely ever homozygous for the factors analysed. This one can understand since

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[1] My earlier hypothesis(2) as to the 3-ds. type being hypostatic is ruled out by the result of the 3 ds. × 5 ds. cross.

the structure of the pelvis, being an internal character, could not be selected on external appearance. Only indirectly could selection be applied to structure of the pelvis through correlations between pelvic structure and external characters of some kind or other amenable to selection.

In the 3-ds. type the acetabulum is shifted forwards along the spine, frequently as far as the level of V. 28, whereas in the 5-ds. type it is shifted backwards to the level of V. 31, and it is reasonable to suppose that these osteological differences react on the position of the body. A forward displacement of the point of support of the body must mechanically result in a more horizontal position, and a backward displacement in a more vertical one. Taking the extreme types of structure (3 ds. and 5 ds.) one can see that in Bantams the body has, as it were, an almost horizontal position, whilst an Orloff cock of the 5-ds. type holds himself more erect. But this can only be perceived in extreme cases and no difference in posture is as a rule perceptible in individuals of the 4-ds. and 5-ds. types, where the displacement of the acetabulum is not considerable.

There is a large proportion of 4-ds. individuals in all the races of poultry dissected, whereas there are marked differences in the distribution of the 5-ds. and especially of the 3-ds. type. The 5-ds. type is most often found in races of big poultry (Faverolles, Cochins); the 3 ds. type belongs specifically to the small races of Bantams, or to those nearly related to them in size (Paduans, etc.).

The *Orloff race* shows a rather high proportion of individuals with the 5-ds. pelvic type. Among 178 pure-bred chickens examined there were 101 N 4 ds. individuals and 77 5 ds. individuals.

Not a single individual of the 4-ds. type had a modified structure. These data show that the combination **Ab** (stimulating the modified 4-ds. type) is very rare among the Orloff poultry owing to the common occurrence of **B** in this breed.

Bantams. Outwardly this type of poultry presents many diverse races characterised by small size and the possession of a whole series of anatomical modifications in their skeleton, but they appear to be generally characterised by possessing **C**. Apparently there are not many homozygous **CC** Bantams, but the heterozygous state **Cc** ensures the spreading of this factor among the small races of poultry. The absence in Bantams of the 5-ds. pelvic type shows that they are homozygous for **A** (or else it makes one suppose the existence of a special inhibiting factor).

Faverolles, Brahmas and *Wyandottes* resemble the Orloff in the frequency of the 5-ds. type. The highest proportion of 4-ds. individuals would appear to be found in *Indian Game, Orpingtons* and *Pavloffs,* but there are so far only very scanty data for these races.

The various structural types of pelvis show no correlation either with the shape or the weight of the egg. Nor is there any genetical correlation with certain of the colour factors discovered in poultry, *e.g.* that for black pigment in the feathers (tifa), or with the factor for leg feathering (asuso) (4).

The genetical relations of the factors and their phenotypes may furnish some indications as to their appearance in the phylogeny of poultry. The factor **C** of the Bantams is certainly the "youngest" among the three factors **A**, **B** and **C**. There exists a hypothesis that the Bantams are derived from other races through the fixing of a mutation (or series of mutations) which altered the general size of the body. Along with this, doubtlessly, the proportions in the skeleton also changed. The shortened 3-ds. type which appeared could in the Bantams be selected on external appearance, because in this race the body has a more horizontal position (a character evidently correlated with the 3-ds. type).

One may suppose the pelvic structure with 5 ds. vertebrae to have been the oldest. The **aB** genotypes (5 ds.) dominated over **ab** (recessive 4-ds. type), and the recessive nature of the 4-ds. type existed until the appearance of the **A** gene, epistatic to **B**. In this way the 4-ds. type became dominant (**AB**), and the relations between the 4-ds. and 5-ds. types became altered in the general population, inclining toward an increase of the number of "new" genotypes **AB** and **Ab**.

It is not of course supposed that peculiarities in pelvic type are the result of only three pairs of factors. Even if the modification of the 4-ds. type described in Section v is not entirely hereditary in nature, there may be other structural modifications dependent upon factors not yet determined.

VIII. SUMMARY.

(1) In domestic races of poultry there are three principal hereditary types of pelvic structure with 3, 4 and 5 dorsosacral vertebrae respectively (see Figs. 1, 2 and 3).

(2) The inheritance of these three types is dependent upon three pairs of factors.

A, a factor stimulating a pelvic structure with 4 ds. vertebrae. In the

absence of **B** it generally leads to a modified form of the 4-ds. type (large proc. transv. of V. 23, Fig. 4 *a*).

a, recessive to **A**, leading to a 4-ds. type in the absence of other dominant factors.

B, a factor stimulating a pelvic structure with 5 ds. vertebrae; hypostatic to **A**, but epistatic to **a** (**aB** = 5 ds.; **AB** = 4 ds. norm.); inhibitory to certain modifications of the 4-ds. type (Fig. 4 *a*, *b*).

b, allelomorph to **B** in the majority of the modified 4-ds. individuals.

C, a factor stimulating the 3-ds. type of pelvis (Fig. 3). Apparently epistatic to **A** and **B** (a certain incompleteness of the epistasis can be observed in the modifications of V. 24 described in Section II, p. 36; the rudiments of proc. transv. on the 24th vertebra).

c, recessive to **C** in individuals of 4-ds. and 5-ds. types.

Genotypes with **ABc**, normal 4-ds. type (Fig. 1); with **Abc**, modified 4-ds. type (Fig. 4 *a*, *b*); with **AaBB** (possibly), a specially modified 4-ds. type (Fig. 4 *c*, *d*; see *x*); with **aBc**, 5-ds. type, with little variation owing to the absence of mutual interaction (antagonism) between **A** and **B**.

Genotypes **aabb**, recessive 4-ds. type; genotypes with **C**, 3-ds. type.

(3) Different pure races of domestic poultry are never uniformly of any given pelvic type. In all races there is a rather high proportion of individuals with 4 ds. vertebrae (the "typical" or normal pelvic structure). Small sized races often show a 3-ds. structure; large ones a 5-ds. one.

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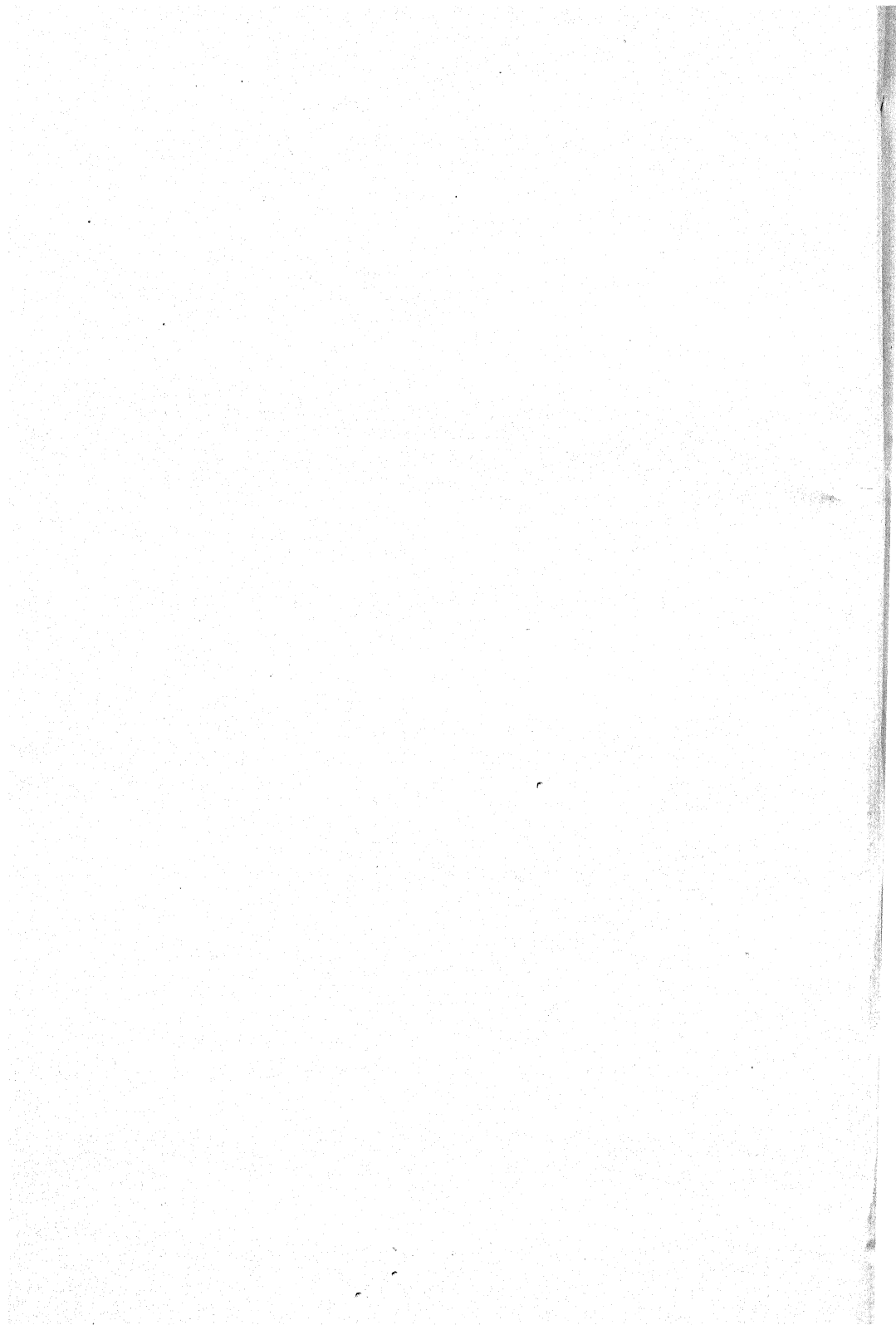
FURTHER STUDIES ON INHERITANCE IN *MATTHIOLA INCANA*. II. PLASTID COLOUR AND DOUBLING.

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(With One Diagram.)

INTRODUCTION.

AT an earlier stage in the investigation[1], as recalled in the last contribution dealing with inheritance in *Matthiola*[2], it was suggested that it might be possible to synthesise a double-throwing (eversporting) strain of Stocks from a non-double-throwing (true-breeding) single if the breeding was carried out in a certain specified way. For if an eversporting single be crossed with pollen from a non-double-throwing single we know that the resulting F_1 singles will be of two kinds, (1) those which yield both singles and doubles in F_2, and (2) those which produce only singles. If for this mating an individual of a d. sulphur-white[3] strain is used as ♀ and a form in which all the pollen carries single cream as ♂, the known heterozygous constitution of the mother plant and the uniform character of the pollen of the male parent enable us, without further breeding tests, *by simple inspection of the plastid colour*, to make a preliminary selection of F_1 individuals to be used in the next step. This will be obvious on reference to the factorial combinations involved in this union set forth on p. 54 in which W = the factor for white (colourless) plastids, and X and Y = the two factors requisite for singleness, X[4] being linked with W in the eversporting, and with Y in the true-breeding single.

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[1] See "On selective partial sterility as an explanation of the behaviour of the double-throwing Stock and the Petunia," *Am. Nat.* L. 496, 1916.

[2] "Further Studies on Inheritance in *Matthiola incana*. I. Sap colour and Surface character," *Journ. Gen.* XIV. 103, 1924.

[3] Here as in the earlier accounts the abbreviations d. and no-d. are used to indicate eversporting and true-breeding singles respectively. A sulphur-white strain yields perpetually about 47 per cent. of white singles, 3 per cent. of white doubles and 50 per cent. of cream doubles.

[4] It is to be noted that as we are unable to distinguish between X and Y the selection of X as the member of the pair supposed to be linked with W is purely arbitrary.

Eversporting sulphur-white ♀ × non-double-throwing cream ♂.

Ovules*   Pollen

15 $\widehat{WX}Y$ ------------------1------------------------------- All $w\widehat{X}Y$
 1 $\widehat{WX}y$ ----------------2-----------------------
 1 wxY --------------3--------
15 wxy ---------4---

* The balance of evidence furnished by the earlier experiments favours the adoption of the gametic ratio 15 : 1 : 1 : 15 for the ovules of eversporting forms rather than 7 : 1 : 1 : 7.

The factorial combinations (3) and (4) produce cream[1] singles all of which may be discarded for all give doubles in some matings. Combinations (1) and (2) give white[1] singles of which those derived from (1), that is, on the average 15 out of every 16, will invariably yield single. Only from (2) will a white be produced from which doubles can be bred in F_2.

On the supposition that in those F_1 whites throwing no doubles the WXY factors are distributed similarly to ovules and pollen we should expect that the F_2 generation from the above cross would include some homozygous individuals of the composition $\widehat{WX}Y\widehat{WX}Y$. Such individuals, though breeding true to singleness, crossed with pollen from an eversporting type would presumably yield offspring *all* of which would once again prove to be eversporting for doubleness. Such was the procedure originally suggested for synthesising the eversporting type[2]. If this homozygous true-breeding F_2 single could be found the back-cross would complete the operation, for *every* individual so derived might be expected to prove eversporting. Experiments on these lines, however, brought to light the fact that such homozygous individuals do not appear in F_2[3] owing to a different distribution of the factors concerned to ovules and pollen respectively in the F_1 cross-bred[4]. The discovery of this sex-linked relation made it desirable both to repeat certain matings between strains of different plastid colour previously carried out, but which at the time had been undertaken primarily with the object of elucidating the mode of inheritance of *doubleness*, and to complete the list of possible combinations, in order to ascertain how the distribution of the W factor in the F_1 individual was affected, according as it was

[1] In order to avoid the repetition of lengthy descriptions, and since in the present account sap colour is nowhere in question, the colours white and cream are here to be taken throughout to refer solely to *plastid* colour irrespective of whether the *sap* in the individuals so described is coloured or colourless.

[2] See footnote 1, p. 53.

[3] Or if they do occur it must be so rarely as to render experiment on the lines suggested impracticable.   [4] *Journ. Gen., loc. cit.*

introduced into the pedigree through the male or female parent and in the linked or unlinked condition. Furthermore, since it now appeared that a *homozygous* individual of the desired constitution ($\widehat{WXY}\widehat{WXY}$) was not obtainable by the method suggested, a *heterozygous* plant would, perforce, have to be utilised in the synthesis of the eversporting type from a non-double-throwing single, a substitution necessitating a restatement of the mode of procedure.

1. INHERITANCE OF THE FACTOR W (COLOURLESS PLASTIDS) IN MATINGS
BETWEEN STRAINS OF DISSIMILAR PLASTID COLOUR.

Mating 1. *d. sulphur-white* ♀ × *no-d. cream* ♂[1].

This union we shall represent factorially thus:

Ovules	Pollen
15 $\widehat{WX}Y$	All $w\widehat{XY}$
1 $\widehat{WX}y$	
1 $wx\,Y$	
15 wxy	

It is obvious that here the F_1 will consist of 4 kinds of cross-breds, and these it will be necessary to treat individually.

(i) F_1 *cross-breds from the* $\widehat{WXY}$ *ovules.* On an average 15 out of every 16 of the F_1 individuals with white plastids thus produced will have the constitution $\widehat{WXY}w\widehat{XY}$. A uniform distribution of the W factor to the ovules and pollen of such cross-breds would lead us to expect the ratio 3 white single : 1 cream single in F_2. In fact, however, the two forms are found to occur in about equal numbers[2]. A similar result was obtained when the ovules of these same F_1 whites were independently tested by fertilising them with pollen known to carry cream. The numbers recorded in these experiments were as follows:

(a) F_1 white singles having the constitution $\widehat{WXY}w\widehat{XY}$ × self;
Case 1. 1 F_2 family comprised 22 white singles and 26 cream singles.
Case 2. 4 F_2 families comprised 39 white singles and 38 cream singles.
Case 3. 1 F_2 family[3] comprised 125 white singles and 128 cream singles.

(b) F_1 white singles having the constitution $\widehat{WXY}w\widehat{XY}$ × d. cream (wxy); 2 F_2 families comprised 102 white singles and 108 cream singles.

[1] See p. 53, footnote 3.
[2] See *Journ. Gen., loc. cit.*
[3] The F_1 individual used to produce this family was one of the two employed in the following back-cross.

On the other hand when the F_1 pollen was independently tested by using these whites as ♂ in a back-cross with d. cream the resulting F_2, as shown below, was all cream.

(c) d. cream $\times$ F_1 whites having the constitution $\widehat{WX}Yw\widehat{X}Y$; 3 F_2 families comprised 29 individuals which were all cream singles.

These results indicate that gametogenesis in these F_1 cross-breds is as follows:

Ovules	Pollen
$\widehat{WX}Y$	All $w\widehat{X}Y$
$w\widehat{X}Y$	

that is to say, *of the two parental combinations* $\widehat{WX}Y$ *and* $w\widehat{X}Y$ *here involved, both are borne by the* F_1 *ovules but only the paternal factor group* $w\widehat{X}Y$ *is carried by the* F_1 *pollen.*

This being so we should not only expect from these F_1 whites when selfed, or back-crossed with cream, that equality of whites and creams in F_2 which was observed, but we should further expect a like result in all succeeding generations, since all the white singles will be of the same constitution. Also, and for the same reason, that in all the F_2 and later generation whites the pollen would again carry only creamness and singleness. This expectation was confirmed experimentally. 19 F_2 whites from the breeding (d. sulphur-white ♀ $\times$ no-d. cream ♂) $\times$ self gave an F_3 of 1074 plants of which 495 were white singles and 579 cream singles (see Table I, p. 71). Another such F_2 white used as ♂ on a d. cream gave 224 offspring, all cream singles.

(ii) F_1 *cross-breds from the* $\widehat{WX}y$ *ovules.* As, so far as we know, all F_1 cross-breds of the same constitution, however derived, behave alike, investigation of the behaviour of cross-breds of this class is postponed until we come to the consideration of the next mating (Mating 2) in which a true-breeding white was employed in place of the sulphur-white strain[1]. Since it has become clear from the results obtained in this latter case that $\widehat{WX}y$ ovules when mated with $w\widehat{X}Y$ pollen give an F_1 which yields on *self*-fertilisation the same results in F_2 and all later generations as those derived from $\widehat{WX}Y$ ovules, it should be mentioned here that the 19 F_2 families cited above as presumably of $\widehat{WX}Y$ origin may conceivably include one or two which are in reality derived from an original $\widehat{WX}y$ ovule. As, however, the result will be the same (1 white single: 1 cream single) in either case the inclusion in the list of a

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[1] See (ii) on p. 58.

family of this origin, if it occurred, would in no wise affect the validity of the result.

(iii) F_1 *cross-breds from the* wxY *and* wxy *ovules.* Both classes of F_1 being homozygous for cream yield only cream in F_2, the former, when selfed, giving all singles, the latter, 3 single: 1 double (see p. 58).

It is evident from the results given under (i) that we cannot obtain from the operation (d. sulphur-white × a no-d. form with cream plastids) × self a homozygous F_2 individual of the composition $\widehat{WX}YW\widehat{X}Y$. Or, if such individuals are produced, it must be so seldom that in setting out to reconstruct from a non-double-throwing single a form eversporting in regard to doubleness and plastid colour (= d. sulphur-white) we must relinquish the idea of starting from a homozygous individual, and must have recourse to a white single which is heterozygous for W and which, although homozygous as regards X and Y, and therefore true-breeding in regard to singleness, is not strictly homozygous so far as the linkage relation of X is concerned.

Mating 2. d. white ♀ × no-d. cream ♂.

The factorial combinations involved in this union will be represented thus:

Ovules	Pollen
15 $\widehat{WX}Y$	All $w\widehat{X}Y$
1 $\widehat{WX}y$	
1 WxY	
15 Wxy	

(i) Precisely similar results were obtained with the cross-breds derived from the $\widehat{WX}Y$ ovules here as in the case of Mating 1, for the F_1 plants are factorially identical with the corresponding F_1 whites obtained by the same operation from a d. sulphur-white and have a similar gametic output. These results were as follows:

(a) 1 F_1 white × self gave 18 F_2 plants all single, 9 of which were white and 9 cream.

(b) 13 F_1 whites used as ♀ in a back-cross with d. cream or d. sulphur-white gave 528 plants in F_2, all single, of which exactly half were white and half cream (see Table II, p. 71).

(c) 1 of these same F_1 whites used as ♂ in a back-cross with no-d. cream gave 280 singles all cream.

It is evident from the above facts that in the case of a d. white, as with a d. sulphur-white, the factor W is not distributed to the pollen of the F_1 cross-breds derived from the $\widehat{WX}Y$ ovules when such a white

is used as ♀ in a mating with any non-double-throwing individual whose pollen carries cream, as ♂[1].

(ii) It remains to consider the F_1 cross-breds derived from the ovules carrying the factor combination for white plastid colour and doubleness. The study of these whites is more easily carried out in the present case than in Mating 1, for a sulphur-white produces on the average only 1 such white in every 16 white F_1, whereas more than half the F_1 plants (on the average 17 in 32) will be of this class when a true-breeding white is employed (see output of ovules by the two strains shown above, pp. 55, 57).

Now these F_1 whites are of three kinds being derived from three different kinds of ovules, viz. those carrying the combinations $\widehat{WX}y$, WxY and Wxy respectively. As regards behaviour, however, we may treat them as constituting only two categories, since if one member of the XY pair is absent the result appears to be the same whichever of the two it may be that is present.

Experiment shows that the same result obtains when the F_1 from $\widehat{WX}y$ (and presumably also WxY) ovules is *self*-fertilised as when the F_1 is derived from sister ovules carrying singleness $(\widehat{WX}Y)$[2]. In the former case, as in the latter, the linkage of X with W in the ♀ gamete and with Y in the ♂ gamete prevents factorial recombination, so that here too the F_1 ovules only carry the two parental combinations while the pollen again carries only the paternal linked couple $\widehat{XY}$. The F_1 gametic output will therefore be represented thus:

Ovules	Pollen
$\widehat{WX}y$ (or WxY)	$w\widehat{XY}$
$w\widehat{XY}$	

Furthermore, as the F_2 whites thus have the same constitution as their F_1 parents, self-fertilisation will yield the same result in all succeeding generations. It follows that the only method which we can employ to distinguish this class of F_1 white carrying doubleness from those sister whites which are homozygous for singleness is to use them as ♀ in a back-cross with d. cream or d. sulphur-white. For the expectation in F_2 from this operation in the present case would be 1 cream single : 1 white double, whereas, as shown above, $\widehat{WX}Yw\widehat{XY}$ whites tested in this way give 1 white single : 1 cream single (see Mating 1 (i) (*b*), p. 55). The actual counts in F_2 from 8 F_1 whites (which were thereby proved to have been derived from $\widehat{WX}y$ (or WxY) ovules) used as ♀ in a back-

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[1] Or if it is occasionally so distributed such an occurrence must be extremely rare.

[2] Since only two classes of F_1 individuals, apart from those derived from Wxy ovules, have so far been distinguished.

cross with either d. cream or d. sulphur-white were 171 cream singles and 180 white doubles, a close approximation to the equality expected on the gametic scheme suggested above. (For an analysis of the individual F_2 families see Table III, p. 71.)

The fact that all singles arising from Xy (and xY) ovules, however bred, whether homozygous for white or cream, or heterozygous for white, breed true to singleness when self-fertilised, or when used as δ even on a double-throwing strain, suggests a necessary precaution where singles of unknown lineage are employed in experiments of this kind. The fact that no doubles are obtained in either case does not suffice to prove that such an individual has the constitution of a true-breeding single. Pollination by an eversporting strain alone affords a crucial test.

(iii) We have finally to examine the results obtained in the case of those F_1 whites which are derived from the other type of ovule carrying doubleness, viz. those which lack both X and Y ($= Wxy$). The F_1 plants employed in the following experiments evidently belonged to this class of white.

	F_2 ex $F_1 \times$ self, F_1 being derived from a Wxy ovule			
(a) Form of mating	White single	Cream single	White double	Cream double
d. marine × no-d. cream	23	8	14	—
	9	3	5	—
d. white × no-d. cream	24	8	6	—
	18	8	7	—
	36	12	25	—
Total	110	39	57	—

The above result suggests that the true ratio is 2 white single : 1 cream single : 1 white double. Such a ratio would obtain if it were the case that the ordinary linkage relations held good as regards the F_1 ovules leading to the appearance of the parental and cross-over factor groups in the proportion of 15 : 1, but that only the parental combinations were carried by the F_1 pollen. On the other hand, the F_2 result

Scheme 1		Scheme 2	
F_1 ovules	F_1 pollen	F_1 ovules	F_1 pollen
15 Wxy	16 Wxy	15 Wxy	15 Wxy
1 wxy	16 $w\widehat{XY}$	1 wxy	1 wxy
1 $W\widehat{XY}$		1 $W\widehat{XY}$	1 $W\widehat{XY}$
15 $w\widehat{XY}$		15 $w\widehat{XY}$	15 $w\widehat{XY}$
	Expectation in F_2		Expectation in F_2
	512 white singles		513 white singles
	256 cream singles		255 cream singles
	256 white doubles		255 white doubles
			1 cream double

would not be very different if crossing-over took place in the pollen as well as the ovules. This will be apparent on comparing the two schemes of gametogenesis shown on p. 59.

It will be seen that on either scheme the expectation in F_2 from $F_1 \times$ self when F_1 is derived from a *Wxy* ovule is 3 single : 1 double and 3 white : 1 cream. The point of difference between the two lies in the slightly different proportion of the singles and doubles which are white and cream respectively. Only experiments carried out on an exceptionally large scale would enable us to decide between the above schemes since the crucial distinction lies in the appearance or non-appearance of one double cream in about every thousand plants. The non-appearance of a double cream in a count of only 206 F_2 plants (see p. 59) cannot therefore be taken to be decisive as between the two schemes in question. The back-cross with a form whose gametes carried doubleness and cream gave, however, an unequivocal result from which it was clear that the gametic output is in accordance with scheme 2, factorial recombination taking place in the pollen as well as the ovules of F_1.

	Number of F_1 plants employed	F_2 ex F_1 individuals derived from *Wxy* ovules			
(b) Form of back-cross mating to test F_1 ovules		White single	Cream single	White double	Cream double
(d. marine × no-d. cream) × d. sulphur-white	9	14	235	293	5
(d. flesh × no-d. cream) × d. sulphur-white combined with d. cream	1	7	42	35	2
Total	21	277	328	7	
Expectation in F_2 on either scheme ...		1ʳ :	15 :	15 :	1
An exact result in the present case would therefore be (calculated to the nearest whole number)		20	296	296	20

	Number of F_1 plants employed	F_2 ex F_1 individuals derived from *Wxy* ovules			
(c) Form of back-cross mating to test F_1 pollen		White single	Cream single	White double	Cream double
d. cream × (d. flesh × no-d. cream)	1	35	43	26	3
d. cream × (d. white × no-d. cream)	4	72	170	101	4
Total	107	213	127	7	
Expectation in F_2 based on Scheme 2		257 :	495 :	255 :	17

The appearance in both of the above operations of the completely recessive form, double cream, establishes the fact of the recombination of the *WXY* factors in both the ovules and pollen of this type of F_1 individual. Although the number recorded for this class in the case of the ovules falls considerably short of the expectation, the uniformity of the linkage relations exhibited in other types of matings leads to the conclusion that the familiar gametic ratio 15 : 1 : 1 : 15 holds good here

as elsewhere, and that the present deficiency is probably due to some accidental irregularity. The fact that in the corresponding test of the F_1 pollen the number of cream doubles recorded is almost an exact result lends support to the view that scheme 2 holds good in the case of both ovules and pollen.

We have now dealt in detail with the several kinds of cross-bred whites which arise in the two matings treated in the preceding pages and we may fitly conclude the account by exhibiting the difference in behaviour of these whites and the method by which they can most easily be identified in a form readily appreciable to the eye, as shown below (w. = white, c. = cream, s. = single, d. = double).

Cross-bred whites from		$F_1 \times$ self	$F_1 \female \times$ d. cream $\male$	d. cream $\female \times F_1 \male$
$\widehat{WX}Y$ ovules	$\times w\widehat{X}Y$ pollen	1 w.s. : 1 c.s.	1 w.s. : 1 c.s.	All c.s.
$\widehat{WX}y$ ovules $\Big\}$ $Wx Y$ ovules	$\times w\widehat{X}Y$ pollen	1 w.s. : 1 c.s.	1 c.s. : 1 w.d.	All c.s.
Wxy ovules	$\times w\widehat{X}Y$ pollen	513 w.s.	1 w.s.	257 w.s.
		255 c.s.	15 c.s.	495 c.s.
		255 w.d.	15 w.d.	255 w.d.
		1 c.d.	1 c.d.	17 c.d.

Mating 3. No-d. cream $\female \times$ d. white $\male$.

In this type of mating all F_1 individuals will presumably have the same constitution, viz. $w\widehat{X}YWxy$. A considerable number of F_2 families derived from this form of mating were raised in some of the early experiments. These records as well as those obtained from more recent breedings are included in the table appearing below.

Form of mating	Number of F_1 plants employed	F_2 ex $F_1 \times$ self			
		White single	Cream single	White double	Cream double
Earlier records from various unions[1]	43	2085	758	1031	1
no-d. cream × d. azure	2	30	9	15	—
no-d. cream × d. flesh	1	143	74	65	1
no-d. cream × d. flesh (another strain)	1	148	75	57	—
no-d. cream × d. white	1	157	55	58	—
no-d. cream × d. marine	10	632	284	283	1
Total		3195	1255	1509	3

When, as in the present case, only three individuals in a population of some thousands are recorded in a class the possibility of experimental error must not be disregarded. If, nevertheless, we accept these three individuals as genuine—and there is no specific ground for doubt, the result taken as a whole points to a gametic output in F_1 analogous to

[1] These totals are compiled from records accumulated prior to 1914, from which, however, some derived from sowings of old seed which might be open to question have been excluded.

that described for the previous type of mating. We shall therefore represent gametogenesis in F_1 as shown below.

F_1 ovules	F_1 pollen
15 $w\widehat{X}\widehat{Y}$	15 $w\widehat{X}\widehat{Y}$
1 $W\widehat{X}\widehat{Y}$	1 $W\widehat{X}\widehat{Y}$
1 wxy	1 wxy
15 Wxy	15 Wxy

This scheme produces the F_2 ratio

513 white single : 255 cream single : 255 white double : 1 cream double

or roughly twice as many white singles as either cream singles or white doubles and about 1 per 1000 of cream doubles. It will be seen that the numbers recorded in some of the later breedings are in very fair accord with the calculated ratio. The less good agreement in the totals for the earlier records is probably attributable in some measure to less successful germination.

Both the pollen and ovules of F_1 were independently tested by appropriate matings and the evidence so obtained goes to support the conclusions based on the results of self-fertilisation. The test of the pollen was made by using F_1 plants as ♂ in a cross with d. cream, a mating which we shall represent factorially thus:

Ovules	Pollen
15 wXY	15 $w\widehat{X}\widehat{Y}$
1 wXy	1 $W\widehat{X}\widehat{Y}$
1 wxY	1 wxy
15 wxy	15 Wxy

On this scheme the above operation would yield the ratio

257 white single : 495 cream single : 255 white double : 17 cream double

or roughly twice as many cream singles as either white singles or white doubles and about 1 cream double in every 60 F_2 plants.

The actual counts were as follows:

Form of mating	Number of F_1 plants employed	White single	Cream single	White double	Cream double
d. cream × (no-d. cream × d. flesh)	4	91	216	136	10
d. cream × (no-d. cream × d. marine)	5	33	86	43	3
	Total	124	302	179	13
where an exact result would be ...	...	155	299	154	10

For the corresponding test of the F_1 ovules F_1 cross-breds were fertilised with a strain whose pollen carried cream and doubleness. This breeding is represented factorially thus:

Ovules	Pollen
15 $w\widehat{XY}$	All wxy
1 $W\widehat{XY}$	
1 wxy	
15 Wxy	

The numbers obtained (see below) were unfortunately small.

	Number of F_1 plants	F_2			
Form of mating	employed	White single	Cream single	White double	Cream double
(no-d. cream × d. tinged white)					
× d. sulphur-white	1	3	13	27	—
(no-d. cream × d. white) × d. cream	1	1	12	12	—
Total	4	25	39	—
where the expected ratio is		1 :	15 :	15 :	1

The evidence obtained from the back-cross in the case of the pollen is sufficiently close to the expected result as to remove any suspicion as to the genuineness of the 3 cream doubles recorded in the F_2 derived from F_1 × self, and may be looked upon as affording full corroboration of the correctness of the gametic scheme suggested above. The absence of any record of cream doubles in the back-cross where F_1 is used as ♀ may well be due to the smallness of the count; although, therefore, this particular result, taken by itself, cannot be regarded as conclusive, considered in conjunction with the others given above it may be looked upon, so far as it goes, as quite in harmony with them.

Mating 4. d. cream ♀ × no-d. white ♂, and (in part) d. sulphur-white ♀ × no-d. white ♂.

These two matings may conveniently be considered together since the results obtained from the sulphur-white ovules carrying cream and

	Number of F_1 plants	F_2 ex F_1 × self*			
Form of mating	employed	White single	Cream single	White double	Cream double
Early records from various unions[1]	13	496	8	9	152
d. cream × no-d. white	2	211	5	9	66
Total	707	13	18	218
Early records from					
d. sulphur-white × no-d. white	76	—	—	21
d. sulphur-white × no-d. white (another strain)	519	14	18	92
Total	595	14	18	113

* Only those F_2 families containing doubles, i.e. those springing from the xy ovules of the eversporting mother strain, are taken into account.

[1] See p. 61, footnote 1.

doubleness will presumably be the same as those given by the corresponding ovules of the true-breeding cream. The data cited on p. 63 include records accumulated in the course of the early experiments together with those obtained from the more recent work.

Gametogenesis in F_1 on the lines with which we have become familiar in the preceding matings would be as follows:

Ovules	Pollen
15 wxy	15 wxy
1 Wxy	1 Wxy
1 $w\widehat{XY}$	1 $w\widehat{XY}$
15 $W\widehat{XY}$	15 $W\widehat{XY}$

This output would yield 3 single : 1 double and 3 white : 1 cream associated in the ratio

737 white single : 31 cream single : 31 white double : 225 cream double

compared with which we have as the total count from the above experiments

1302 white singles, 27 cream singles, 36 white doubles, 331 cream doubles.

An independent test of the F_1 pollen by a back-cross with d. cream as ♀, a mating represented thus:

Ovules	Pollen
15 wXY	15 wxy
1 wXy	1 $w\widehat{XY}$
1 wxY	1 Wxy
15 wxy	15 $W\widehat{XY}$

gave the following results

Form of mating	Number of F_1 plants employed	White single		Cream single		White double		Cream double
d. cream × (d. cream × no-d. white)	2*	79		39		1		47
where the expectation is		495	:	257	:	17	:	255
and where therefore an exact result would be		80		42		3		41

(calculated to the nearest whole number)

* Being the same individuals as were self-fertilised in the preceding experiment.

This agreement is sufficiently close as to leave little doubt that the gametic output in F_1 corresponds with the scheme given above.

We have now to take account, where d. cream was employed in the original cross as the ♀ parent, of the F_2 families which are all single, which will have sprung from those ovules of the ♀ parent which carry singleness. 15 such all-single F_2 families derived from selfed sister plants to the 15 F_1 cross-breds which gave mixed families (see p. 63) comprised

altogether 471 individuals, *all singles with white plastids*. The F_1 individuals producing these families will have the constitution $wXYW\widehat{X}Y$ and we may infer from the above result that although these F_1 plants are heterozygous for plastid colour *none of their pollen carries cream*. Ovules carrying white and cream are no doubt produced in equal numbers so that we may presumably represent the gametic output of these individuals thus (see statement at foot of p. 75):

Ovules	Pollen
15 wXY	All $W\widehat{X}Y$
1 $\widehat{WX}Y$	
1 $w\widehat{X}Y$	
15 $W\widehat{X}Y$	

We get from this union the exact converse of the result obtained when the paternal pollen carries $\widehat{X}Y$ associated with w. As previously stated (pp. 56 and 57), when the $\widehat{WX}Y$ ovules of a sulphur-white or of a true-breeding white are fertilised with $w\widehat{X}Y$ pollen the pollen of the resulting F_1 *all carries cream*. Hence in this latter case, since cream is recessive to white, F_2 is mixed white and cream, whereas in the present mating F_2 is all white.

Mating 5. No-d. white ♀ × d. cream ♂ or d. sulphur-white ♂.

A repetition of this type of mating recently carried out gave the results listed under (*a*), those obtained in earlier experiments appear under (*b*).

Form of mating	Number of F_1 plants employed	F_2 ex F_1 × self			
		White single	Cream single	White double	Cream double
(*a*) no-d. red × d. cream	1	131	4	6	48
no-d. red × d. sulphur white	1	187	5	4	65
no-d. white × d. sulphur-white	1	86	3	2	33
Total		404	12	12	146
(*b*) no-d. azure × d. cream	1	88	5	1	30
no-d. red × d. sulphur-white	3	152	2	4	68
no-d. white × d. sulphur-white	6	364	6	8	105
Total		604	13	13	203
From both sets of experiments taken together we get	1008	25	25	349	

The F_1 cross-breds will here all be alike and will have the same constitution ($W\widehat{X}Ywxy$) as those F_1 individuals which in the reciprocal cross (Mating 4) were derived from ovules carrying doubleness. Hence the gametic output will be similar and will yield as before (see p. 64) 3 single : 1 double and 3 white : 1 cream associated in the ratio

737 white single : 31 cream single : 31 white double : 225 cream double

which in the present case would work out to

1013 white singles, 42 cream singles, 42 white doubles, 309 cream doubles

where

1008 white singles, 25 cream singles, 25 white doubles, 349 cream doubles

were actually recorded.

Both the ovules and pollen of the F_1 were separately tested by a back-cross, d. cream being employed in this operation in the case of the pollen, and d. cream and d. sulphur-white, both pure-bred and of cross-bred origin, in the case of the ovules. These results are shown below.

Form of mating	Number of F_1 plants employed	F_2 White single	Cream single	White double	Cream double
(no-d. white × d. cream) × d. cream	2	166	10	3	84
(no-d. red × d. cream) × d. cream	3	42	2	2	55
Another breeding of the same type	4	52	—	—	57
(no-d. white × d. sulphur-white) × d. sulphur-white	3	104	3	3	111
(no-d. white × d. sulphur-white) × d. sulphur-white (another strain)	6	190	7	16	243
(no-d. white × d. sulphur-white) × d. sulphur-white (another strain)	5	70	4	1	69
(no-d. red × d. sulphur-white) × d. sulphur-white	3	84	2	5	82
(no-d. red × d. sulphur-white) × (d. flesh × d. cream)	3	81	3	1	91
(no-d. white × d. sulphur-white) × (d. flesh × d. cream)	6	64	—	3	52
Total		853	31	34	844

The gametic combinations involved in the above operation will be as follows:

Ovules	Pollen
15 $W\widehat{XY}$	All wxy
1 $w\widehat{XY}$	
1 Wxy	
15 wxy	

the expectation being 1 single : 1 double and 1 white : 1 cream associated in the ratio

15 white single :   1 cream single :   1 white double :   15 cream double.

826 white single : 55 cream single : 55 white double : 826 cream double

would in the present instance therefore be an exact result where

853 white singles, 31 cream singles, 34 white doubles, 844 cream doubles

were actually recorded.

It will be noticed that both in this and in the preceding experiment

there is a decided deficiency of the two smaller categories. This, however, was not the case in the back-cross test of the F_1 pollen where the observed result was in almost exact agreement with expectation (see below).

Form of mating	Number of F_1 plants employed	White single	Cream single	White double	Cream double
			F_2		
d. cream × (no-d. red × d. cream)	1	5	4	—	2
d. cream × (no-d. white × d. sulphur-white)	3	16	4	1	7
d. cream × (no-d. white × d. sulphur-white) (another strain)	2	54	33	2	31
Total		75	41	3	40

Represented in terms of factors this experiment will appear thus:

Ovules	Pollen
15 wXY	15 wxy
1 wXy	1 Wxy
1 wxY	1 $w\widehat{XY}$
15 wxy	15 $W\widehat{XY}$

Here the expectation is 47 single : 17 double and 1 white : 1 cream associated in the ratio

495 single white : 257 cream single : 17 white double : 255 cream double.

77 white single : 40 cream single : 3 white double : 39 cream double

would therefore in the present case be an exact result where

75 white singles, 41 cream singles, 3 white doubles, 40 cream doubles

were, in fact, recorded.

In view of this result and of the general harmony observable in the whole series of experiments I am disposed to think that the discrepancies observed in the two preceding experiments are accidental and that the output of the F_1 ovules in the present case, like that of the pollen, conforms to the general scheme.

Mating 6. d. white ♀ × d. cream ♂.

This type of mating has been carried out frequently and always with the same results. The F_1 white singles behave, so far as plastid colour and doubleness are concerned, like a pure-bred sulphur-white, yielding offspring in the ratio 15 white single : 1 white double : 16 cream double. This we should expect since the factorial combinations involved are the same in the two cases. The descendants of these cross-breds continue eversporting in all later generations, and the pollen of every individual carries only cream and doubleness. In illustration of these two latter statements we may cite the following experiment.

Form of mating	Number of F_2 plants employed	F_3 ex F_2 × self		
		White single	White double	Cream double
(d. white × d. cream) × self	3	19	2	13
(d. tinged white × d. cream) × self	4	89	6	139
(d. sulphur-white × d. cream) × self	4	62	2	101
Total		170	10	253

As this experiment was undertaken with other ends in view the F_3 plants were not all brought to flower, but the preponderance of doubles among which only a small percentage are white, and the absence of cream singles leave no doubt as to the character of the F_2 individuals.

Direct proof that all the pollen of these eversporting cross-breds carries cream and doubleness is afforded by an experiment in which two such plants were employed as ♂ in a back-cross with d. cream. 198 plants thus obtained were flowered *and all were cream*, 80 being single and 118 double.

Mating 7. d. cream ♀ × d. white ♂.

Here, as in the preceding mating (6), the F_1 and all later generations may be expected to be eversporting in regard to doubleness but the position as regards plastid colour is different, the factorial combinations involved in this cross being as follows:

Ovules	Pollen
15 wXY	All Wxy
1 wXy	
1 wxY	
15 wxy	

Obviously the F_1 will all be white. Six F_1 singles derived from various unions of the above type, selfed, gave the expected preponderance of doubles in F_2. Singles and doubles together numbered 526 plants *and all were again white*. And since each white single which appears will have the same factorial constitution, F_3 and all succeeding generations will also be white, notwithstanding that every single will be heterozygous for W. Direct proof was obtained by using F_1 cross-breds (four) as ♂ in a back-cross with d. cream. 132 singles and doubles were raised *and all were white*. From these experiments we learn that although by crossing d. white × d. cream we can obtain an artificial sulphur-white, *i.e.* a form eversporting as regards plastid colour as well as doubleness, we cannot achieve the same result by making the reciprocal cross. This latter cross once made, the cream colour exhibited by the mother plant disappears from the genealogical tree for all time, unless re-introduced, or unless some new condition arises to unmask it. On the other hand, when these cross-breds are used as ♀ in the same back-cross we may

expect a result on the same lines as that got with the reciprocal hybrid, viz. the ratio 15 cream single : 16 white double : 1 cream double. A family raised in this way gave 9 cream singles and 13 white doubles; the count was no doubt on too small a scale to show a cream double.

2. SYNTHESIS OF AN EVERSPORTING TYPE FROM NON-DOUBLE-THROWING SINGLES.

With the knowledge gained from the various preceding experiments we are now in a position to attempt the solution of our original problem, viz. the reconstruction of eversporting types, and above all a sulphur-white, from non-double-throwing singles. In order to accomplish this we must begin with an XY single, *i.e.* one in which X is not linked with Y, obtain from it by an appropriate mating the type of single which yields no doubles, and then proceed from such singles to construct anew the eversporting individual. It has become clear that the simplest way by which this reconstruction can be accomplished, if we desire an individual of the sulphur-white pattern, that is to say one eversporting as regards both plastid colour and doubleness, is to carry out the following operations. (1) A sulphur-white single which, it may be noted, is indistinguishable in appearance from a true-breeding white, is fertilised with pollen from a pure-bred non-double-throwing cream. On an average 15 out of 16 of the F_1 whites so produced will have the constitution $\widehat{WXY}w\widehat{XY}$. Such plants throw no doubles; they, and all succeeding generations descended from them, produce only single offspring. (2) An F_1 white selected at random is fertilised with pollen from an eversporting pure cream or from a sulphur-white. If now, as is likely, our chance selection has fallen upon one of these $\widehat{WXY}w\widehat{XY}$ and therefore non-double-throwing whites (and we have no choice but to leave the selection to chance for we are unable to recognise these whites by inspection or to identify them by any other test), the F_2 raised from the back-cross will be mixed white and cream since half the pollinated ovules carry white and half cream. All the F_2 whites thus obtained will have the constitution $\widehat{WXY}wxy$. Thus in constitution they are identical with the original pure-bred sulphur-white. (3) These synthesised sulphur-whites and all succeeding generations derived from them, self-fertilised, behave like the original pure-bred strain. The experimental results obtained in this manner were as follows. In one case a sulphur-white individual as ♀ was crossed with a non-double-throwing cream form (half-hoary tinged white with cream plastids). One of the resulting F_1 whites, selected at random,

was fertilised with cream-carrying pollen (that of a cross-bred from the mating d. flesh × d. cream). This operation produced 56 white singles and 60 cream singles *but no doubles*. Nine of these F_2 whites were selfed and all proved to be of the eversporting type in regard both to doubleness and plastid colour, the F_3 consisting of white singles and cream doubles with a small percentage of white doubles but no cream singles. In another case the start was made with an eversporting F_3 out of an original cross between a double-throwing true-breeding white as ♀ and a d. cream as ♂. This plant was pollinated with a non-double-throwing cream from the same strain as that used in the first case. This operation yielded 16 white singles and 13 cream singles *but again no doubles*. One of these F_2 whites, when selfed, behaved as a typical sulphur-white giving a mixed offspring of 34 white singles, 31 cream doubles, 3 white doubles and another double which was lost before the flowers opened. In this instance the experiment was carried a step further and another generation was raised from 4 of these F_3 singles. Again the same result was obtained, showing that an eversporting race had been synthesised which would behave in all succeeding generations like the pure-bred sulphur-white. The actual numbers recorded in these 4 families and in the 9 families of the preceding experiment are shown in Table IV, p. 71.

We have now dealt with the results as regards singleness and doubleness and white and cream plastids produced by the several factorial combinations occurring in the various possible matings between strains of dissimilar plastid colour. It is worthy of note that although the F_2 result from F_1 × self in several cases is 3 single : 1 double and 3 white : 1 cream we do not in any of these instances obtain exactly the simple ratio 9 white single : 3 cream single : 3 white double : 1 cream double owing to linkage between the two factors for singleness. Also, that in certain other cases where doubleness does not enter into the problem and where therefore this simplification would supposedly lead to the appearance of the anticipated 3 white : 1 cream, this result does not obtain owing to the linkage of one of the two factors for singleness with the factor determining plastid colour, the issue in this case being still further complicated by the sex-limited distribution of this linked pair.

Finally, we may recall that when both parents are of similar plastid colour matings between d. and no-d. strains as a rule give an F_2 ratio from F_1 × self of 3 single : 1 double in the mixed families, whichever way the cross be made. In the earlier experiments[1] a very marked

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[1] *Journ. Gen.* I. 338 and 352, and Tables IV and V. On p. 352 the 46 families to which reference is made in Mating 11 are wrongly printed as the F_3 instead of the F_2 generation.

deficiency of doubles occurred in some matings between certain individuals which, used in other matings, gave the expected result. No recurrence of such extreme cases has been observed in the later work, and the suggestion that in these earlier matings we were dealing with an additional pair of factors $X'Y'$ producing singleness remains a possibility, but one of which, as yet, there is no further confirmation.

TABLE I.		TABLE II.		TABLE III.	
Analysis of 19 F_3 families; see p. 56.		*Analysis of 13 F_2 families; see p. 57.*		*Analysis of 8 F_2 families; see p. 59.*	
White single	Cream single	White single	Cream single	Cream single	White double
137	141	47	23	45	48
75	88	41	34	41	44
66	58	28	35	41	41
35	56	27	41	18	15
33	36	27	26	15	8
22	20	23	21	7	14
20	27	19	33	3	5
20	22	17	7	1	5
20	21	10	8	171	180
12	17	9	12	*Exp.* 1 : 1	
9	11	8	10		
8	19	6	9		
8	7	2	5		
7	9	264	264		
6	11	*Exp.* 1 : 1			
5	14				
4	11				
3	9				
5	2				
495	579				
Exp. 1 : 1					

TABLE IV.

Analysis of 9 F_3 families (see p. 70) and of 4 F_4 families (see p. 70)

	Total single	Total double	White single	White double	Cream double
F_3	8	16	6	—	16
	15	32	14	3	26
	16	18	16	—	15
	36	43	21	3	31
	37	52	29	1	45
	25	40	20	—	40
	18	20	12	2	17
	37	53	35	1	50
	21	35	18	2	32
F_4	47	58	47	2	56
	45	42	41	1	35
	20	29	13	—	26
	12	17	12	1	16
Exp.	15 :	17 :	15 :	1 :	16

SUMMARY.

1. The present experiments entirely confirm the conclusions arrived at in the earlier work in regard to the inheritance of singleness and doubleness, viz.

(i) That singleness is dependent upon the presence of two factors (X and Y) and that in the absence of either or both the flower is double.

(ii) That in the non-double-throwing strains X is linked with Y (shown thus $\widehat{XY}$) but that in the eversporting strains the two factors are not thus linked (shown thus XY); and that whereas $\widehat{XY}$ can be carried by both ovules and pollen XY is only borne by ovules.

(iii) That matings between two strains, both eversporting, yield the same proportion of singles and doubles in F_1 and all succeeding generations as the two pure-bred eversporting parent strains, *i.e.* 15 single : 17 doubles.

(iv) That matings between non-double-throwing and eversporting singles yield as a rule 3 single : 1 double in the mixed F_2 families derived from $F_1 \times$ self.

2. They also fully support the view put forward in the earlier accounts that white plastid colour (dominant) is due to the presence of a single factor (W) in the absence of which the plastids are cream (recessive).

3. They further show that matings between forms of dissimilar plastid colour yield orderly and predictable results, but that in these unions the gametic output of the F_1 cross-breds and consequently the composition of the F_2 generation differ according as double-throwing or non-double-throwing strains are employed, and according as the factor for white plastid colour is introduced into the pedigree on the male or female side, and in the unlinked condition or linked with one of the factors for singleness.

4. The results obtained in F_2 from the various possible unions coming under the above heads can be accounted for by the application and extension of the scheme of factorial interrelations adopted in the earlier accounts. From the whole of the evidence now available it appears:

(i) That if the factors for singleness (X and Y) are introduced into the pedigree in the linked condition ($\widehat{XY}$), whether on the male or female side, they (? invariably) remain linked in the gametes.

(ii) That when X and Y occur in the unlinked condition and in association with W one of the two factors (assumed to be X) is linked with W ($\widehat{WX}$).

(iii) That the coupled factors $\widehat{WX}$ like the couple $\widehat{XY}$ remain linked in gametogenesis; but whereas $\widehat{XY}$ can be borne both by ovules and pollen and can therefore be introduced from, and transferred to, either side of the pedigree, $\widehat{WX}$ is only carried by ovules, can therefore only be introduced from the female side, and having been so introduced cannot be transferred to the pollen.

(iv) That when a union involves the combination of $\widehat{WX}$ and $\widehat{XY}$ no factorial recombination takes place in gametogenesis since different factor pairs are linked in the two parents and there are consequently no corresponding free allelomorphs. Heterozygotes of this constitution produce only the two parental types of gametes, and of these only one, the paternal (see (iii)), will be borne by the pollen. If heterozygous for W these individuals will yield 1 white : 1 cream on selfing.

(v) That when a union involves the combination $\widehat{WX}Ywxy$ both the parental ($\widehat{WX}Y$ and wxy) and the cross-over ($\widehat{WX}y$ and wxY) combinations are borne by the ovules in the ratio of 15 : 1, but only the paternal combination is carried by the pollen (see (iii)). Such heterozygotes also yield 1 white : 1 cream when selfed. Also, that these same relations hold when w is introduced in association with XY and W with xy ($wXYWxy$). In this case, however, the offspring of the selfed heterozygotes are all white.

(vi) That, on the other hand, in heterozygotes having the constitution $\widehat{WX}Ywxy$ or $w\widehat{XY}Wxy$ and in the reciprocal cross-breds $wxy\widehat{WX}Y$ and $Wxyw\widehat{XY}$ both parental and cross-over combinations in the ratio of 15 : 1 occur in the pollen as well as in the ovules, hence any form of these matings yields 3 white : 1 cream in F_2 as well as 3 single : 1 double. Owing, however, to the above-mentioned linkage relations the four forms white single, cream single, white double, cream double are never obtained in the simple ratio of 9 : 3 : 3 : 1.

5. The gametic output based upon the above factorial relations obtaining in F_1 from the various possible unions between white and cream true-breeding and eversporting strains is shown in the scheme set forth on p. 74 together with the expectation resulting therefrom in F_2.

Scheme of factorial constitution and gametic output in terms of the factors for singleness and doubleness and plastid colour.

XY = singleness; xy, Xy and xY = doubleness; W = white plastids; w = cream plastids.

Pure strains.

	no-d. white $W\widehat{XY}W\widehat{XY}$		no-d. cream $w\widehat{XY}w\widehat{XY}$		d. white* $W\widehat{XY}Wxy$		d. cream $w\widehat{XY}wxy$		d. sulphur-white* $W\widehat{XY}wxy$	
Zygote										
Gametes	Ovules	Pollen	Ovules	Pollen	Ovules	Pollen	Ovules	Pollen	Ovules	Pollen
	All $W\widehat{XY}$	All $W\widehat{XY}$	All $w\widehat{XY}$	All $w\widehat{XY}$	15 $W\widehat{XY}$	All Wxy	15 $w.XY$	All wxy	15 $W\widehat{XY}$	All wxy
					1 $W\widehat{Xy}$		1 $w.Xy$		1 $W\widehat{Xy}$	
					1 WxY		1 wxY		1 wxY	
					15 Wxy		15 wxy		15 wxy	

* As we are unable to distinguish between Xy and xY the assumption that in the eversporting white and sulphur-white strains W is linked with X rather than with Y is purely arbitrary.

F₁ cross-breds.

w.s. = white single; c.s. = cream single; w.d. = white double; c.d. = cream double; A. = All; N. = None.

Parents	Factor combinations involved in the case of those ovules which carry singleness	Factor combinations involved in the case of those ovules which carry doubleness	F₁ gametes Ovules	F₁ gametes Pollen	F₂ ex F₁ × self single : double	white : cream	w.s. : c.s. : w.d. : c.d.	F₂ ex F₁ × d. cream w.s. : c.s. : w.d. : c.d.	F₂ ex d. cream × F₁ w.s. : c.s. : w.d. : c.d.
d. white♀ × no-d. cream♂ d. sulphur-white♀ × no-d. cream♂ d. white♀ × no-d. cream♂	W͡XY × wX͡Y	Wxy × wX͡Y	W͡XY / wX͡Y	A. wX͡Y	A. N. : 3 : 1	A. N. : 3 : 1	A. N. : 513 : 255 : 255 : 1	A. N. : 1 : 15 : 15 : 1	A. N. : 257 : 495 : 255 : 17
d. cream♀ × no-d. white♂	wXY × WX͡Y	Wxy × wX͡Y	15 wX͡Y / 1 [1 wX͡Y / 1 WX͡Y] / 15 WX͡Y	A. WX͡Y	A. N.	A. N.	A. N.	1 : 1 : 0 : 0	1 : 1 : 0 : 0
d. cream♀ × no-d. white♂ d. sulphur-white♀ × no-d. white♂	wxy × WX͡Y		15 wxy / 1 Wxy / 1 wX͡Y / 15 WX͡Y	15 wxy / 1 oX͡Y / 15 WX͡Y	3 : 1	3 : 1	737 : 31 : 31 : 225	15 : 1 : 1 : 15	495 : 257 : 17 : 255
no-d. white♀ × d. cream♂ no-d. white♀ × d. sulphur-white♂	WX͡Y × wxy		15 WX͡Y / 1 wX͡Y / 1 Wxy / 15 wxy	15 WX͡Y / 1 wX͡Y / 1 Wxy / 15 wxy	3 : 1	3 : 1	737 : 31 : 31 : 225	15 : 1 : 1 : 15	495 : 257 : 17 : 255
no-d. cream♀ × d. white♂	wX͡Y × Wxy		15 wX͡Y / 1 WX͡Y / 1 wxy / 15 Wxy	A. Wxy	3 : 1	3 : 1	513 : 255 : 255 : 1	1 : 15 : 15 : 1	257 : 495 : 255 : 17
d. white♀ × d. cream♂ d. sulphur-white♀ × d. cream♂	W͡XY × wxy		15 W͡XY / 1 WX̂y / 15 wxy	A. wxy	15 : 17	1 : 1	15 : 0 : 1 : 16	0 : 15 : 0 : 1	0 : 15 : 0 : 17
d. cream♀ × d. white♂	wX͡Y × Wxy		15 wxy / 1 W͡Xy / 1 wx̂Y / 15 W͡XY	A. wX͡Y	15 : 17	1 : 1	15 : 0 : 17 : 0	15 : 0 : 17 : 0	15 : 0 : 17 : 0
d. white♀ × no-d. cream♂ d. sulphur-white♀ × no-d. cream♂	W͡Xy × wX͡Y		W͡Xy / wX͡Y	A. wX͡Y	A. N. : 1 : 1	A. N. : 1 : 1	1 : 0 : 1 : 0	0 : 1 : 1 : 0	A.

...netic combinations which have not been identified but which are not excluded on the present scheme are enclosed in brackets. *If* recombination takes place when a wX͡Y ovule ...ised with WX͡Y pollen the F₁ gamete carrying WXY will presumably behave as though W and X were linked, and is therefore represented as WX͡Y.

6. The relation between the mode of linkage of the WXY factors and the gametic output is illustrated in a manner readily appreciable to the eye in the accompanying diagram (p. 77).

7. The fact that no further instances have been recorded in the more recent work such as occurred in the earlier experiments of families showing so considerable a deficiency of doubles as for it to be improbable that this deficiency could be attributed to some accidental irregularity, leaves the earlier suggestion that we are concerned in these cases with a second pair of factors, $X'Y'$, producing singleness, still unconfirmed, though it would seem to be the simplest formula which meets these exceptional cases.

8. The linkage relations between the WXY factors and those governing the distribution of these factors in the linked and the unlinked condition to the male and female gametes respectively having become clear, the reconstruction of an eversporting strain from a non-double-throwing single could at once be carried out. The method of procedure is the same as that suggested in the earlier accounts, but whereas it had previously been imagined that the end operation would result in a homogeneous population every individual of which would prove eversporting, we now know that only half the plants so obtained will have this character, though it will be possessed by *all their descendants* in all succeeding generations. When a sulphur-white is taken as the starting-point in these operations the eversporting singles, which form half the F_2 generation from a rightly selected F_1, can be identified by their plastid colour without additional experimental test.

9. The difference in behaviour between the eversporting and true-breeding strains appears to result from:

(i) The linkage of X with W in the eversporting and with Y in the true-breeding strains.

(ii) The ability of the eversporting strains to produce ovules carrying the linked couple $\widehat{WX}$ and the unlinked couple XY. They are, however, unable to form male gametes carrying either of these combinations. Pure-bred homozygous non-double-throwing singles are incapable of forming gametes, male or female, carrying either combination although ovules and pollen alike can carry W in the unlinked and X and Y in the linked ($\widehat{XY}$) condition.

Diagram illustrating the mode of linkage of the WXY factors in different cases and the resulting gametic output.

ov. = ovules, p. = pollen.

True-breeding singles

no-d. white   no-d. cream

Eversporting singles

d. white   d. cream   d. sulphur-white

ov.   p.   ov.   p.

Cross-bred singles

no-d. white × d. cream
$W\widehat{X}Y × wxy$

no-d. cream × d. white
$w\widehat{X}Y × Wxy$

d. white × no-d. cream

d. cream × no-d. white

(1) $W\widehat{X}Y$ ovules × $wx\widehat{Y}$

(2) Wxy ovules × $wx\widehat{Y}$

(1) wXY ovules × $WX\widehat{Y}$
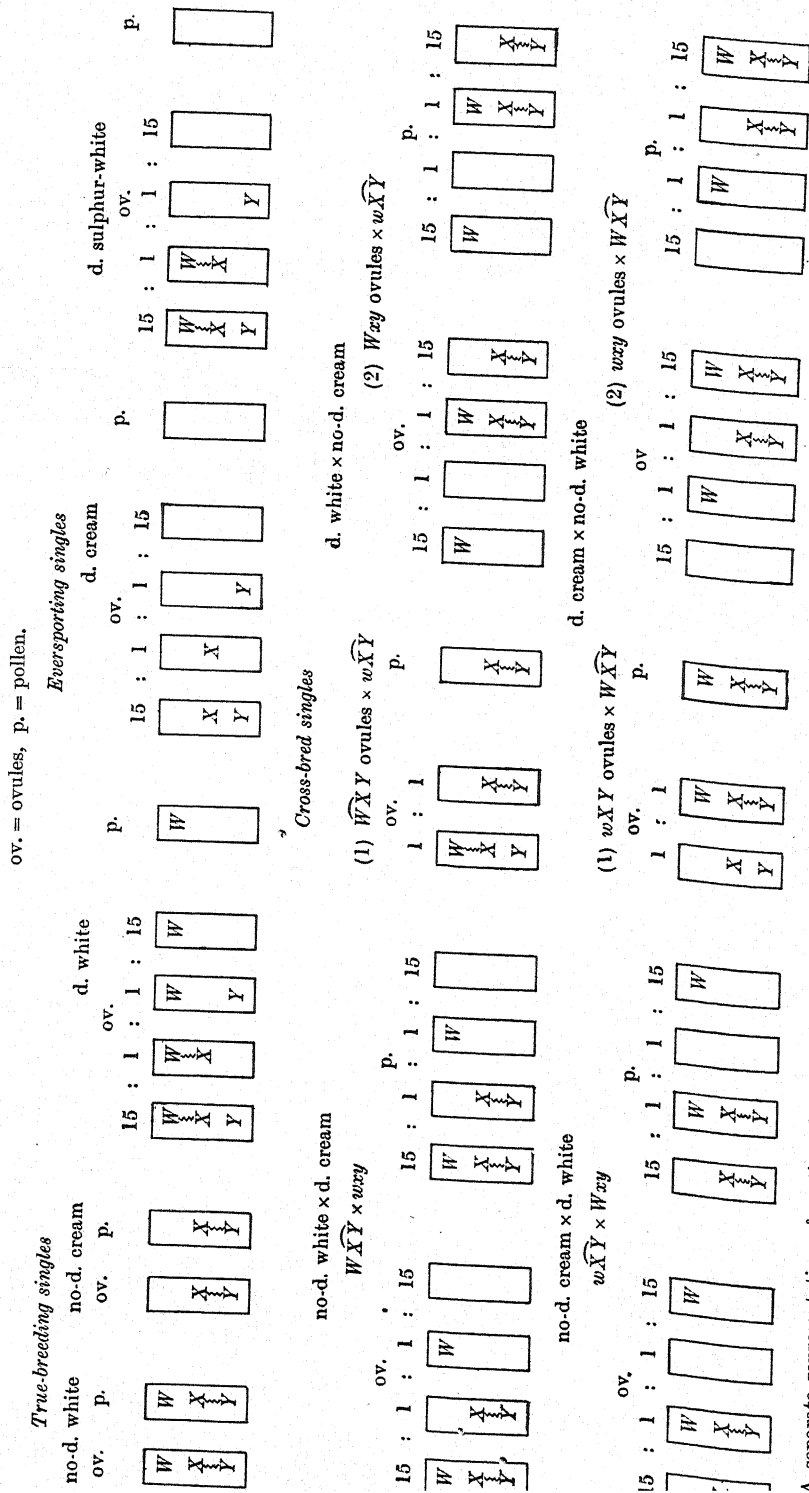
(2) wxy ovules × $WX\widehat{Y}$

A separate representation of matings in which d. sulphur-white is employed is unnecessary since the results where the WXY and WXy ovules are concerned will be the same as when d. white is used, and similarly where the wxY and wxy ovules and its pollen are in question, as when d. cream is used. For [...] gametic combinations not represented here see text.
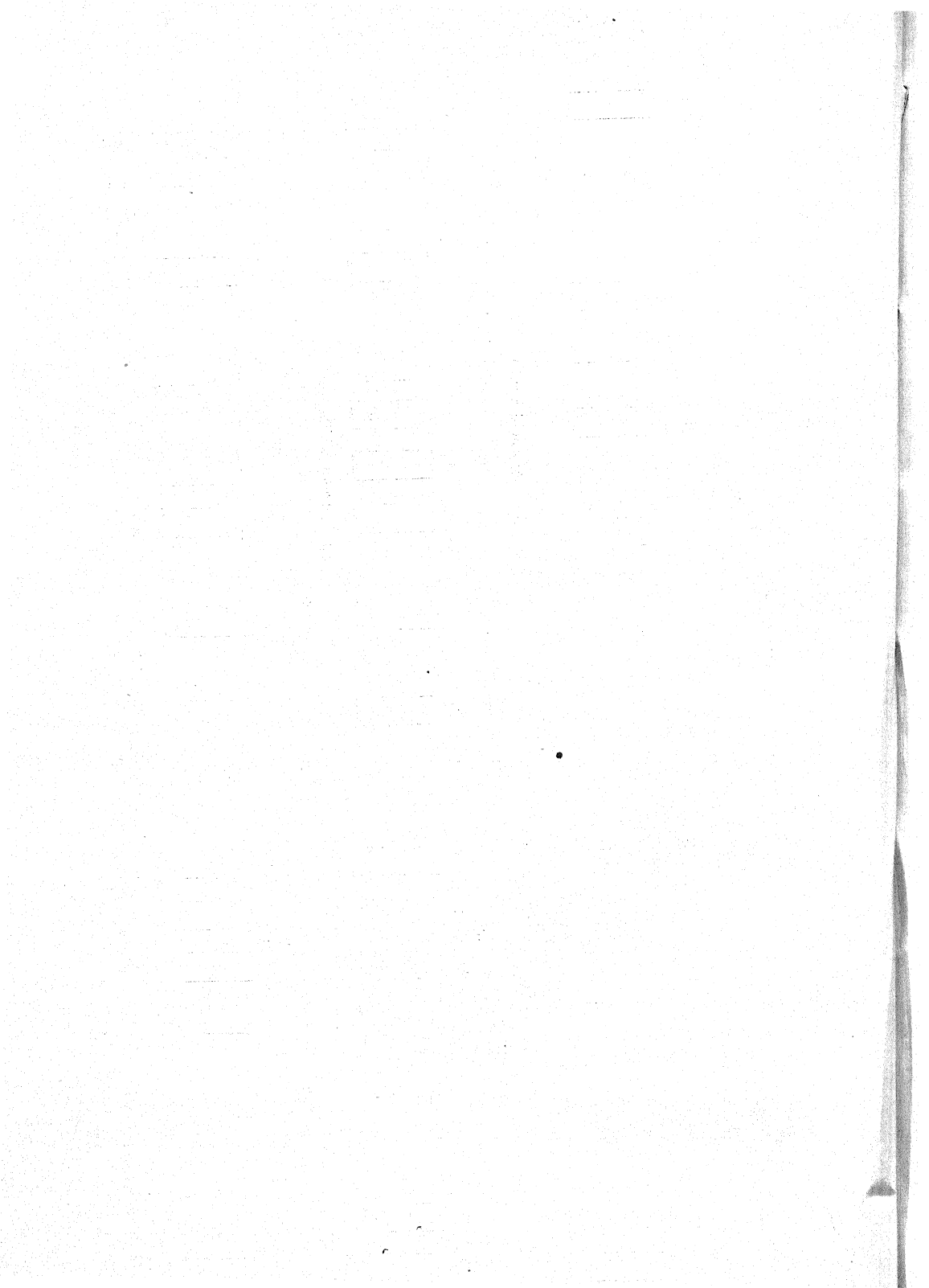
THE ESTIMATION OF LINKAGE FROM THE OFFSPRING OF SELFED HETEROZYGOTES.

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(With Two Text-figures.)

1. INTRODUCTION.

THE method of measuring the linkage between two factors by crossing the double heterozygote back to the double recessive, supplies a direct measure of the proportion of the "recombination" classes to the total count; no statistical methods are necessary in examining the data beyond expressing the number of offspring in the two recombination classes as a percentage of the total offspring counted.

It is frequently preferable, in view of the labour saved, to obtain a progeny by selfing the heterozygote. In such cases unlinked factors should give four classes of offspring in the ratio $9 : 3 : 3 : 1$, and linkage will manifest itself by an excess of the first and last classes, if both dominant genes came from the same grandparents (Coupling), and by an excess of the second and third classes if they came from different grandparents (Repulsion).

It should be noticed that progenies of this kind are influenced by linkage both in the male and in the female gametogenesis. If therefore the recombination percentages are different in the two sexes, or if it is desired to study such differences as may exist, the method of backcrossing offers a considerable advantage. The method of selfing is capable of detecting such a sexual difference, as will be shown more clearly below, but is relatively insensitive for this purpose. If on the contrary, as is more usually the case, the object is to detect any linkage which may exist, the method of selfing is not in itself unsuitable. Its advantages would be more obvious if suitable methods had from the first been used in estimating the linkage value, for the method has suffered some unmerited disrepute from the extremely inefficient methods which have been usually employed in this process of estimation.

2. THE QUANTITATIVE EFFECTS OF LINKAGE.

A heterozygote **AaBb** of two factors **A** and **B** will produce gametes of four types **AB**, **Ab**, **aB** and **ab**. If the factors are unlinked these will be produced in equal numbers; if linked, the classes **AB** and **ab**

will appear with a frequency different from the classes **Ab** and **aB**. In general, whatever the origin of the heterozygote may have been, we may represent by q the proportion of the gametes belonging to the two latter classes, and by p $(= 1 - q)$ the proportion belonging to the two former. The same will be true both in male and in female gametogenesis, but since the value of p may be different for the two sexes, we may use p for the proportion in the female gametogenesis, and p' for the corresponding proportion in the male gametogenesis.

We shall then have gametes capable of mutual fertilisation produced in the following proportions:

	AB	**Ab**	**aB**	**ab**
Ovules	$\frac{1}{2}p$	$\frac{1}{2}q$	$\frac{1}{2}q$	$\frac{1}{2}p$
Pollen	$\frac{1}{2}p'$	$\frac{1}{2}q'$	$\frac{1}{2}q'$	$\frac{1}{2}p'$

It is evident that the fraction of offspring in the double recessive class is $\frac{1}{4}pp'$, and since this with either of the two singly recessive classes must make up a quarter of the whole, these must each have a fraction $\frac{1}{4}(1 - pp')$, leaving for the class which is recessive in neither factor, the balance of $\frac{1}{4}(2 + pp')$. The whole effect of linkage is therefore expressible in terms of the single quantity pp' which we may hereafter designate by x. If the recombination fraction is the same in both sexes, it will be obtained as the square root of x for repulsion, and as $1 - \sqrt{x}$ for coupling.

If the value of $\sqrt{x}$ for repulsion progenies differs significantly from $1 - \sqrt{x}$ for coupling progenies, then the recombination values for the two sexes will be significantly different; they may be found by solving the equations

$$pp' = x_1,$$
$$(1 - p)(1 - p') = x_2,$$

which lead to a quadratic equation of which the roots are p and p'. The method as mentioned above is insensitive compared with the use of back-cross data, and when the two recombination values are found it does not tell us which belongs to which sex.

3. The Estimation of Linkage.

The values of x may be estimated, from the observed numbers in the four classes, in a considerable variety of ways. We shall discuss the advantages enjoyed by some estimates over others derived from the same data. It will be made clear that some of the advantages are universal and of such a kind as we should always wish to obtain, others are adventitious and are only realised in special circumstances. In particular

the advantage of the class of estimates technically called *efficient statistics* over all other estimates is universal, while among the efficient group we may find some better than others in special cases. The comparisons may be conveniently made on a numerical example (Carver[7]), showing linkage between the sugary factor in maize and a factor for white base leaf. The case was one of repulsion, and the numbers of seedlings counted were

Starchy		Sugary		
Green	White	Green	White	Total
1997	906	904	32	3839

As a representative group of methods we may take the following:

(a) Additive method, also called Emerson's formula; x is calculated from the sum of the first and fourth classes.

(b) Weighted mean method; x is calculated from an alternative linear function of the frequencies.

(c) Product method; x is calculated from the products of the first and fourth classes and of the second and third classes; equivalent to the method of Bridges[2].

(d) Method of maximum likelihood, first applied to this problem by Haldane[1] who derives a process of approximation which can only lead to this solution, from the condition that χ^2 shall be a minimum.

(e) Method of minimum χ^2.

The estimate obtained by each of these methods is made to conform to the criterion of consistency, which simply involves the condition that if the sample of observations were increased without limit, the method would give the correct value to any required degree of approximation. This may be ensured by writing, instead of the observed frequencies, quantities in the theoretical ratio $2 + x : 1 - x : 1 - x : x$ which will be approached as the sample is increased indefinitely. If then the observed frequencies are a, b, c and d in a total of n offspring, the first method (a) will consist in equating $a + d$ to its expected value $\frac{n}{4}(2 + 2x)$; the resulting equation may be written

$$nx = a - b - c + d,$$

from which x, and thence the recombination percentage, may be immediately calculated. In our example we have

$$3839x = 219,$$
$$x = \cdot057046,$$

giving a recombination percentage of 23·88.

For this estimate the comparison of expected with observed frequencies is as follows:

| | Starchy | | Sugary | | |
	Green	White	Green	White	Total
Expected (m)	1974·25	905	905	54·75	3839
Observed	1997	906	904	32	3839
Difference (δ)	+22·75	+1·0	−1·0	−22·75	0
δ^2/m	0·262	0·001	0·001	9·453	9·717

The value of the measure of discrepancy χ^2 is 9·717 and for two degrees of freedom this will occur by chance less than once to each 100 trials. The conclusion indicated is that the number of sugary-white seedlings has been depressed by some cause additional to linkage. It will be shown that this conclusion is unwarranted, although it follows inevitably if this method of estimation is employed.

For each method of estimation it is possible to calculate the variance due to errors of random sampling; since the variance is not always the same for different methods of estimating the same quantity, these calculations have an important bearing upon the adequacy of any particular method in making use of the information supplied by the data. The variance of our estimate of x by method (a) is

$$\frac{1 - x^2}{n} \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(1),$$

giving a standard error for x of ·01601, or of 3·373 per cent. in the recombination percentage.

For method (b) we shall take the expression $a - 3b - 3c + 9d$, which, by the criterion of consistency, must be equated to $n(4x - 1)$. In fact, the equation for x is

$$4nx = 2a - 2b - 2c + 10d,$$

or, numerically $3839x = 173·5,$

$$x = \quad ·045194,$$

giving a recombination percentage of 21·26.

The comparison of expected with observed frequencies is now

| | Starchy | | Sugary | | |
	Green	White	Green	White	Total
Expected (m)	1962·875	916·375	916·375	43·375	3839
Observed	1997	906	904	32	3839
Difference (δ)	+34·125	−10·375	−12·375	−11·375	0
δ^2/m	0·593	0·117	0·167	2·983	3·860

The measure of discrepancy found in this case is only 3·860, a value which will be exceeded by chance in more than 15 per cent. of trials.

This value therefore indicates that there is no significant departure from the expected frequencies, contrary to the conclusion drawn from method (a).

The random sampling variance of the estimate of x by method (b) is

$$\frac{1 + 6x - 4x^2}{4n} \quad\dots\dots\dots\dots\dots\dots\dots(2),$$

giving a standard error for x of ·00907, or of 2·133 for the recombination percentage.

It will be noticed that the standard error of the estimate by method (a) is materially larger than that by method (b). The latter method therefore makes use of a larger fraction of the information supplied by the data, and gives in consequence a better estimate of linkage.

In method (c) we have the equation for x

$$\frac{x\,(2 + x)}{(1 - x)^2} = \frac{32 \times 1997}{906 \times 904},$$

a quadratic equation of which the positive solution is

$$x = ·035645.$$

The comparison of expected with observed frequencies becomes

	Starchy		Sugary		
	Green	White	Green	White	Total
Expected (m)	1953·711	925·539	925·539	34·211	3839
Observed	1997	906	904	32	3839
Difference (δ)	+43·289	−19·539	−21·539	−2·211	0
δ^2/m	0·9592	0·4125	0·5012	0·1429	2·0158

The measure of discrepancy has fallen still further, and is now scarcely greater than its average value 2·0; the apparent defect of the double recessive class was evidently due wholly to the method used to estimate linkage.

The random sampling variance of x by method (c) is found to be

$$\frac{2x\,(1 - x)\,(2 + x)}{n\,(1 + 2x)} \quad\dots\dots\dots\dots\dots\dots\dots(3),$$

giving a standard error for x of ·00584, or of 1·545 for the recombination value.

The formula (3) is of special interest, for we shall find exactly the same expression for the random sampling variance of the two remaining methods of selection, namely the method of maximum likelihood and the method of minimum χ^2. Now it has been proved[3] that the method of maximum likelihood will in all cases supply a solution of which the

random sampling variance is as small as possible; consequently the group of solutions which have the same random sampling variance is of special importance in that they may be said to convey the whole of the available relevant information supplied by the sample. Such solutions are termed efficient statistics. Other solutions, such as are exemplified by methods (a) and (b), convey only a determinate fraction of the available information; their efficiency is measured by the fraction which they convey, and this fraction may be measured by the ratio which the minimum random sampling variance bears to the actual variance appropriate to each statistic. Fig. 1 shows for all values of $\sqrt{x}$ from 0 to 1 the actual efficiency of the methods of solution (a) and (b).
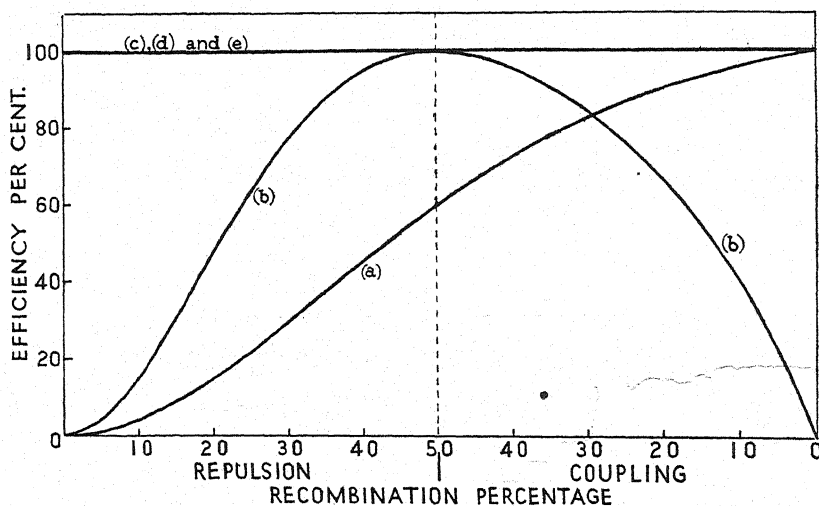
Fig. 1.

Since no statistic can exist of which the random sampling variance is less than the expression (3), this expression effectively specifies the amount of information, relevant to the value of x, which the data contain. Actually the quantity of information may be consistently measured, in large samples, by the inverse expression

$$I = \frac{n\,(1 + 2x)}{2x\,(1 - x)\,(2 + x)}.$$

By the aid of this quantitative measure of the amount of information, it will be seen that the efficiency of any statistic is simply the ratio of the quantity of information which it conveys, to the quantity of information latent in the data from which it was derived. To obtain the

corresponding quantitative measure of the amount of information relevant to the recombination fraction, the above expression should be multiplied by $4x$. The expression then shows the extent of the advantage of coupling over repulsion experiments for all recombination values; or, equally, the ratio of the numbers of seedlings to be counted to obtain equal precision.

It will be seen that each of the methods (a) and (b) attains 100 per cent. efficiency in special circumstances. Method (a) is perfectly efficient when $x = 1$, that is for close linkage measured by coupling. It is probable that this fact has led to its use in other circumstances, such as close linkage measured by repulsion, for which it is totally unfitted. For the case of our example its efficiency is less than 13 per cent., and its use for estimating linkage is equivalent to disregarding nearly seven-eighths of the data. Method (b) is perfectly efficient at $\sqrt{x} = \cdot 5$, or in the important group of cases in which crossing over is so frequent that linkage can scarcely be detected; it supplies a quick and useful test of the *significance* of apparent linkage, namely that the square of $(a - 3b - 3c + 9d)$ should exceed $36n$, but is a bad method for the estimation of close linkage. Method (c), like methods (d) and (e), is perfectly efficient for all values of x.

The method (d) of maximum likelihood [3, 4] consists in maximising a quantity which can be written down by multiplying each observed number by the logarithm of the expectation. Thus

$$a \log (2 + x) + b \log (1 - x) + c \log (1 - x) + d \log x$$

is a maximum, if

$$\frac{a}{2 + x} + \frac{d}{x} = \frac{b + c}{1 - x},$$

leading to the quadratic equation for x

$$nx^2 - (a - 2b - 2c - d)\, x - 2d = 0,$$

or
$$3839x^2 + 1655x - 64 = 0,$$

of which the positive solution is

$$x = \cdot 035712,$$

the recombination percentage is 18·898, and the comparison of expected with observed frequencies is

	Starchy		Sugary		
	Green	White	Green	White	Total
Expected (m)	1953·775	925·475	925·475	34·275	3839
Observed	1997	906	904	32	3839
Difference (δ)	+43·225	−19·475	−21·475	−2·275	0
δ^2/m	·9563	·4098	·4983	·1510	2·0154

It will be seen from the fact that the expectations differ from those of method (c) by only about one-sixteenth of a seedling in each class, that this solution agrees closely with that already obtained. It has indeed been proved that, at least in the theory of large samples, all efficient solutions will be equivalent. We may therefore anticipate that a third scarcely distinguishable solution will be obtained by the procedure (e) of making χ^2 a minimum.

The measure of discrepancy χ^2 may be expressed as an explicit function of x in the form

$$\chi^2 = \frac{4}{n} \left(\frac{a^2}{2+x} + \frac{b^2}{1-x} + \frac{c^2}{1-x} + \frac{d^2}{x} \right) - n;$$

the condition that this should be a minimum leads to a quartic equation for x, of which the solution in our example is

$$x = \cdot035785,$$

giving a recombination value of 18·917.

The comparison of expected with observed frequencies is

	Starchy		Sugary		
	Green	White	Green	White	Total
Expected (*m*)	1953·845	925·405	925·405	34·345	3839
Observed	1997	906	904	32	3839
Difference (δ)	+43·155	−19·405	−21·405	−2·345	0
δ^2/m	·9532	·4069	·4951	·1601	2·0153

The results obtained by the five methods employed are summarised in the following table:

	Recombination value obtained	Standard error $x = \cdot0357$	χ^2	Method
(a)	23·880	4·268	9·7170	Sum of complementary classes
(b)	21·260	2·348	3·8600	Weighted mean
(c)	18·880	1·545	2·0158	Product
(d)	18·898	1·545	2·0154	Maximum likelihood
(e)	18·917	1·545	2·0153	Minimum χ^2

To make these comparable the standard errors have now all been calculated for the same value of x.

4. ADVANTAGES OF DIFFERENT METHODS OF ESTIMATION.

The principal advantages of the efficient group of statistics are:

(i) They provide better estimates of the linkage. The value 23·88 per cent. obtained by method (a) is not only a bad estimate from the data; it is an estimate which the data, when properly interpreted, firmly repudiate. Indeed it differs from the value indicated by the data

by more than three times the real standard error. The observations are in fact inconsistent with any real value much over 22 per cent., and to derive a value of over 23 per cent. from them is totally to misinterpret their evidence.

(ii) It has been shown(5) that χ^2 measures the discrepancy between observation and hypothesis only when efficient statistics are used. If the estimation is carried out by less efficient methods, then χ^2 will be affected by errors of fitting of the same order as the errors of random sampling, and will not provide any real test of the hypothesis. In the present example the values of χ^2 found by using efficient statistics show that there is no sign of a significant departure from expectation; while using method (a) the investigator is inevitably led to believe that very significant irregularities are present. This misleads him in two ways. On the one hand, he seems to have evidence of differential viability of the genotypes concerned, and on the other, whether he ascribes the apparent irregularities to differential viability or not, a cautious man will not place much reliance on estimates of linkage in which one or more of the frequencies have apparently been disturbed from their theoretical relationship. Observations therefore which are in reality free of differential viability, or other causes of error, may, by the mere inefficiency of the method of estimation, be discredited, or regarded as evidence that differential mortality occurs.

(iii) The efficient group of statistics agree so closely among themselves that no practical difference is to be expected between the conclusions drawn by using the different methods. This property follows from the theorem that the correlation between any two efficient statistics tends to $+ 1.0$ as the size of the sample is increased. With tolerably large samples it can therefore be expected that all efficient methods will be practically equivalent.

In ease of calculation, (c) and (d) which lead to quadratic equations are distinctly superior to (e) which leads to a biquadratic. Of the quadratics that of the maximum likelihood method is the simpler to write down, and should never take more than a few minutes to solve. Method (c) is, however, capable of simplification by tabulating the values of the product ratio for different recombination percentages. For this purpose we give such a table (p. 91) of these ratios and their logarithms. Thus in our example the ratio is ·078025, and the table at once shows that the recombination per cent. is nearly 19. Direct interpolation gives 18·876. Alternatively, some will find it more convenient to use the logarithm 8·89223 for interpolation in the column of logarithms provided;

this gives 18·882; both values being amply near enough to the exact value 18·880. Logarithmic interpolation is much the more accurate for high linkage.

In the numerous genetic problems analogous to the one treated as a detailed example in this note, the method of maximum likelihood may be conveniently used to obtain at least one method of solution of known efficiency. If other methods are also efficient this will only be known by comparing the standard sampling error with that of the maximum likelihood solution. In general it may be said that such differences as exist between the different efficient statistics are always to the theoretical advantage of the maximum likelihood solution.

A good example of this may be illustrated in the present case by a previously unsuspected connection between the measure of discrepancy and the maximum likelihood solution.

It is now well known that although in the distribution of a given number of individuals among four classes, there are three degrees of freedom, yet if, as in the present problem, the expected numbers have been calculated from those observed by means of an adjustable parameter (x), then only two degrees of freedom remain in which observation can differ from hypothesis(6). Consequently the value of χ^2, calculated in such a case, is to be compared with the values characteristic of its distribution for two degrees of freedom. This principle has been disputed, but the commonsense considerations upon which it was based have since received complete theoretical verification. In the present instance we can in fact identify the two degrees of freedom concerned. For the observed numbers in each class will be entirely specified if we know:

  (i)   the number in the sample;
  (ii) the ratio of starchy to sugary plants;
  (iii) the ratio of green to white base leaf;
  (iv) the intensity of linkage.

Now if the expected series agrees in items (i) and (iv), it can only differ in items (ii) and (iii), and these will be completely specified by the two quantities p and q defined by

$$p = a + b - 3c - 3d,$$
$$q = a - 3b + c - 3d,$$

specifying the ratios by linear functions of the frequencies.

The mean values of p and q will be zero, and the random sampling variance of each will be $3n$.

In the absence of linkage their deviations will be independent, but if linkage is present the mean value of pq may be found to be

$$- 3n \frac{1 - 4x}{3},$$

or, the correlation between p and q is

$$\rho = - \frac{1 - 4x}{3}.$$

The simultaneous deviation of p and q from zero will therefore be measured by

$$Q^2 = \frac{1}{3n} \left\{ \frac{1}{1 - \rho^2} (p^2 - 2\rho pq + q^2) \right\}$$

$$= \frac{3}{8 (1 - x) (1 + 2x) n} \left\{ p^2 + q^2 + \frac{2}{3} (1 - 4x) \ pq \right\}.$$

This expression, which of course depends upon x, is a quadratic function of the frequencies; in this it resembles χ^2, and on comparing term by term the two expressions it appears that

$$\chi^2 = Q^2 + \frac{1}{I} \left\{ \frac{a}{2 + x} - \frac{b + c}{1 - x} + \frac{d}{x} \right\}^2,$$

where I is the quantity of information contained in the data, as defined in Section 3.

This identity has two important consequences; first that $\chi^2 = Q^2$ for the particular value of x given by the equation of maximum likelihood, and for no other value. At this point then, even for finite samples, the deviations between observation and expectation represent precisely the deviations in the single factor ratios. The large value of χ^2 obtained by solution (a) could be seen at once to be fictitious from the fact that p and q, being $+ 95$ and $+ 87$ respectively, are not large compared to their standard error $\sqrt{3n} = 107 \cdot 3$.

The second point is that for any value of x, χ^2 is the sum of two positive parts of which one is Q^2, while the other measures the deviation of the value of x considered from the maximum likelihood solution; this latter part is the contribution to χ^2 of errors of estimation, while the agreement of observation with hypothesis is measured by Q^2 only.

Fig. 2 shows the values of χ^2 and Q^2 over the region covering the three efficient solutions.

The contact of the graphs at the maximum likelihood solution, makes it evident why the solution based on minimum χ^2 should be of no special interest, although χ^2 is a valid measure of discrepancy between observation

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and hypothesis. As the hypothetical value, x, is changed, the value of Q^2 changes, and, although this change is very minute, it gives the line a sufficient slope to make an appreciable shift in the point of contact. The solution of the minimum χ^2 equation will in fact always be slightly biassed in whichever deviation tends to diminish Q^2, although the deviations to which Q^2 is due have nothing to do with linkage.

Finally there is one advantageous property possessed by the product formula (c) which deserves the attention of geneticists. If one of the factors concerned, but not the other, has an appreciable effect upon viability, this circumstance will affect the two products equally, and
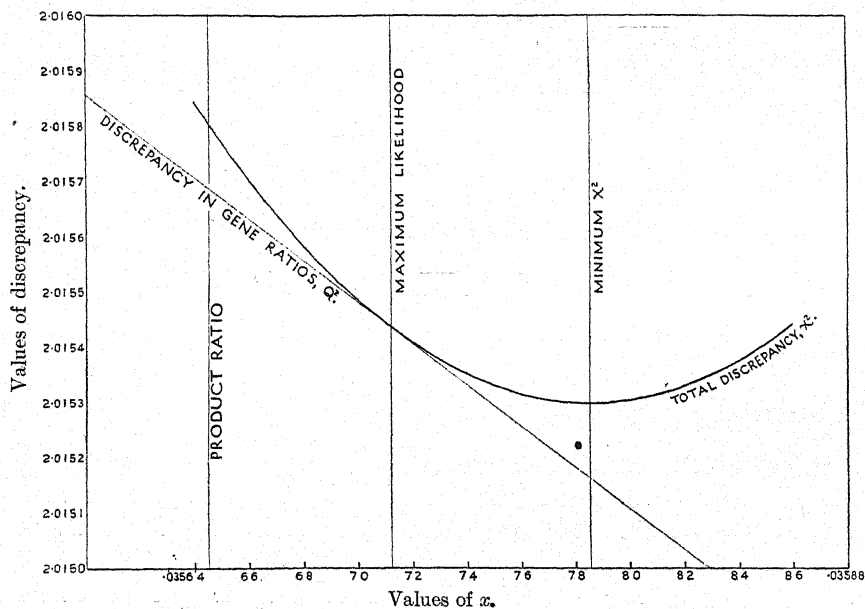
Fig. 2.

have no influence upon their ratio. Consequently the product formula is uninfluenced by such a source of error. Of course, it is also unaffected if both factors affect viability provided the percentage loss due to each factor is independent of the presence of the other, but we should require positive and independent evidence that this is so, in cases in which both factors affect the viability.

5. SUMMARY.

Five methods of solution are given of the statistical problem presented by typical linkage data. The example chosen shows the various errors

into which the use of inefficient statistics leads. Of the efficient methods the method of maximum likelihood possesses the advantage that it may be applied directly to any analogous problem, and is related in a previously unsuspected way to the measure of discrepancy χ^2. The product ratio method, for using which a table is provided, enjoys the practical advantages of other efficient solutions, and is in addition unaffected by differential viability, if this is caused by one factor only. The method of minimum χ^2, unlike the other two, is laborious in computation, and seems to possess no special theoretical interest.

Table for use of product formula.

Re-combi-nation %	Coupling Ratio of products bc/ad	Log + 10	Repulsion Ratio of products ad/bc	Log + 10	Re-combi-nation %	Coupling Ratio of products bc/ad	Log + 10	Repulsion Ratio of products ad/bc	Log + 10
1	·$0^3$1356	6·13216	·$0^3$20005	6·30114	26	·1467	9·16646	·16077	9·20621
2	·$0^3$5516	6·74158	·$0^3$80080	6·90352	27	·1616	9·20855	·17581	9·24504
3	·$0^2$1262	7·10116	·$0^2$18041	7·25626	28	·1777	9·24959	·19185	9·28296
4	·$0^2$2283	7·35847	·$0^2$32128	7·50688	29	·1948	9·28961	·20894	9·32002
5	·$0^2$3629	7·55979	·$0^2$50314	7·70169	30	·2132	9·32875	·22715	9·35631
6	·$0^2$5318	7·72572	·$0^2$72652	7·86125	31	·2328	9·36704	·24654	9·39189
7	·$0^2$7366	7·86724	·$0^2$99210	7·99656	32	·2538	9·40454	·26721	9·42685
8	·$0^2$9793	7·99091	·013007	8·11418	33	·2763	9·44133	·28987	9·46123
9	·01262	8·10099	·016532	8·21833	34	·3002	9·47748	·31268	9·49510
10	·01586	8·20030	·020508	8·31192	35	·3258	9·51302	·33767	9·52849
11	·01954	8·29099	·024946	8·39700	36	·3532	9·54798	·36431	9·56147
12	·02375	8·37566	·029861	8·47510	37	·3823	9·58245	·39270	9·59406
13	·02832	8·45211	·035268	8·54738	38	·4135	9·61643	·42300	9·62634
14	·03347	8·52460	·041183	8·61472	39	·4467	9·64999	·45531	9·65831
15	·03915	8·59272	·047625	8·67784	40	·4821	9·68315	·48980	9·69002
16	·04540	8·65706	·054616	8·73732	41	·5199	9·71595	·52663	9·72151
17	·05240	8·71933	·062177	8·79363	42	·5603	9·74841	·56598	9·75280
18	·05972	8·77616	·070334	8·84717	43	·6034	9·78058	·60806	9·78395
19	·06787	8·83165	·079112	8·89824	44	·6494	9·81249	·65307	9·81496
20	·07670	8·88482	·088542	8·94715	45	·6985	9·84417	·70126	9·84588
21	·08628	8·93591	·098654	8·99411	46	·7510	9·87563	·75289	9·87673
22	·09663	8·98512	·109480	9·03933	47	·8071	9·90692	·80824	9·90754
23	·10780	9·03262	·121070	9·08304	48	·8671	9·93806	·86763	9·93833
24	·11984	9·07860	·133450	9·12532	49	·9313	9·96908	·93142	9·96915
25	·13279	9·12317	·146670	9·16634	50	1·0000	10·00000	1·00000	1·00000

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THE INHERITANCE OF DWARFING IN
GAMMARUS CHEVREUXI.

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(With Two Text-figures.)

CONTENTS.

(1) INTRODUCTION.

THE physiology of inheritance is among the more important genetic problems awaiting thorough investigation at the present time. The distribution and behaviour of the genes, and the characters for which they are responsible, have been studied in a variety of forms, but the developmental processes by which these characters are produced have so far received but little attention. Goldschmidt's well-known study of sex-determination in *Lymantria* (1923) was the first definite advance in this branch of the subject. It has been followed (Goldschmidt, 1927) by important work on the physiological aspect of a number of other genetic phenomena.

Holometabolous insects, however, do not provide the best material for work of this kind, since growth and differentiation practically cease when they assume the imaginal phase. A more suitable form has been found in the Amphipod *Gammarus chevreuxi* Sexton, in which a number of genetic factors are already known. Of these a considerable proportion control the *rate* at which certain characters develop, and throw light on the physiological processes concerned in the action of the genes (Huxley and Ford, 1925; Sexton and Pantin, 1927; Ford and Huxley, 1927). In the latter paper will be found some discussion of the occurrence and significance of such factors, and references to the previous work on

Gammarus, which has largely been due to the careful researches of Mrs Sexton.

The object of the present paper is to give an account of a new rate-factor. This controls the body growth, and the age at which sexual maturity is reached.

I am greatly indebted to Prof. J. S. Huxley, who has given me his most valuable help and advice, not only in this investigation but during the whole time that I have been engaged in studying genetic problems. The present work has been carried out in the Department of Zoology and Comparative Anatomy, Oxford, and I should like to express my best thanks to Prof. E. S. Goodrich, F.R.S., for the facilities he has afforded me. I am much indebted to the Department of Scientific and Industrial Research for a number of grants. I have also to thank Mrs Sexton, of the Marine Biological Laboratory, Plymouth, for supplying stocks of *Gammarus* and for much information concerning the species.

(2) Phenotypic and Genotypic Dwarfing in *Gammarus*.

During a number of years' work on *Gammarus*, dwarf forms have repeatedly turned up in the stocks. These were isolated on several occasions, but the dwarfing proved to be environmental, and no evidence of the inheritance of this character was obtained until recently.

Actually, such dwarf specimens were successfully mated with the normal form fourteen times. These crosses gave normal offspring which were inter-bred to give an F_2 generation of 189 individuals, not counting those which died before a difference in growth could be detected. Of these all were normal except one. Unfortunately the single dwarf did not live until sexual maturity, but it doubtless represented a chance example of phenotypic dwarfing similar to the original specimens. Matings between two dwarfs were obtained four times; these gave an F_1 of 59 specimens, and a large F_2 of which 163 were examined. No dwarfs appeared in either generation.

Somewhat similar dwarfing has been encountered by Sexton and Wing (1916), who suggest that it may be due to bacterial infection. Bacteria have frequently attacked stocks at Oxford and, if they do not kill them, they often retard their growth. The isolated dwarfs described above were probably produced in this way, but it is rather curious that the remaining animals in the same pots escaped the infection.

Mr C. S. Elton has noticed that the growth of *Gammarus duebenii* can be retarded very considerably by feeding on dead leaves only, without green food such as *Ulva* or lettuce. In this way he was able to produce

sexually mature *G. duebenii* of approximately the same size as *G. chevreuxi*, although it is normally a much larger species. Further work on these lines is desirable, especially in view of the remarkable effect of size on eye-colour (see Section 4), and of the possibility of obtaining pairings between the larger and smaller *Gammarus* species. In this connection Mr Elton was able to show that *G. duebenii* and *G. chevreuxi* would not pair even when the barrier of size was removed. In the present case, however, *Ulva* was always supplied and differences in food cannot have accounted for the dwarfing.

Some time ago several dwarf specimens appeared among the offspring of a female from stock. These were being used for certain body-measurements and not for genetic experiments, so the numbers were not recorded, but two of the dwarfs were mated and found to breed true. Further matings made with the specimens thus obtained established the fact that, in this case, dwarfing is inherited, and behaves as a single recessive factor, which may be called **g** (= slow growth, see p. 96). The results are summarised in the following tables:

TABLE I.

F_2 *segregation.*

	Numbers obtained		
Family	Normal	Dwarf	Total
D1P3a1a1	12	3	15
D2P3a1a1	15	5	20
D1H1a1a1–3	30	8	38
D2H9a2a1	10	3	13
D2J2a1a1–3	24	6	30
	91	25	116

Ratio—Normal : Dwarf = 3·64 : 1.

TABLE II.

R_2 *segregation.*

	Numbers obtained		
Family	Normal	Dwarf	Total
D1H2a1D3	10	8	18
D1J3a1D1a1–3	21	17	38
D1P2a1D3	4	6	10
D1C8a1D3a1–3	28	24	52
	63	55	118

Ratio—Normal : Dwarf = 1·15 : 1.

It will be seen from the above figures that the expected 3 : 1 and 1 : 1 ratios are approximately realised. The divergence from them, which

is slightly greater in the former case, is not statistically significant. This may be demonstrated by means of the χ^2 distribution (Fisher, 1925)[1]. There is an excess of normal over dwarf specimens in both ratios, doubtless due to the decreased viability of the dwarfs, which are more delicate than the normal form. Fortunately it is easy to separate the two classes among fairly young animals, but if the number of sexually mature individuals were counted the disparity would be considerably increased. Indeed it has been a matter of some difficulty to obtain enough sexually mature specimens for this investigation.

The F_1 generation was normal in all the above crosses, with one exception. In the F_1 family D1H1a1 (which was inter-bred to give the F_2 family D1H1a1a1–3) one dwarf specimen appeared, out of a total of twelve which lived long enough for the types to be separated. This fortunately survived, and was mated to a **g.g** individual; an F_1 of eight specimens was obtained, all normal. It thus appears that the exceptional dwarf in F_1 was an example of the environmental dwarfing already mentioned, which occurs sporadically among the stocks.

It may be noted that characters, such as the dwarfing described in the present paper, which may either be of a phenotypic or a genotypic nature, are of some interest from an evolutionary point of view (Robson, 1928; and see also Goldschmidt, 1927).

(3) The Nature of the Genotypic Dwarfs.

The action of the recessive factor described-in the last section is in reality to *retard the growth* of the specimens homozygous for it. Ultimately they grow into normal-sized males and females. The proportions of the parts in these specimens have not yet been investigated in detail, though work on this point is proceeding. Superficially, however, they appear normal in this respect. The term *dwarf* is therefore not strictly applicable, and I have therefore chosen the symbol **g** (= slow growth) for the new factor, the wild-type (**G**) being used to denote normal growth-rate.

In comparing the growth of *Gammarus* strains the head-length may be taken as a convenient standard. This has been measured from the side, as a straight line taken from the tip of the slight projection above

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[1] The numbers in the F_2 generation are: normal 91, dwarf 25, total 116. From these figures $\chi^2 = 0.736$. There is one degree of freedom (*i.e.* given a fixed total, one class to which a random value could be assigned). From the table of χ^2 the value of P (the probability that χ^2 shall exceed any specified value) in the present case lies between 0.50 and 0.30. Since $P = 0.05$ may be taken as the value below which a real discrepancy is indicated, the departure from the expectation is certainly not significant. (For the formula and tables used see Fisher, *l.c.*) Similarly the R_2 generation gives $\chi^2 = 0.542$, for which P again lies between 0.50 and 0.30.

the base of the first antennae to the posterior end of the head along the dorsal surface, seen by transparency through the overlap of the next segment. All the specimens used in the above matings were kept at a constant temperature of 23° C. Under these conditions the average head-length at the time of extrusion from the brood-pouch is 0·26 mm.
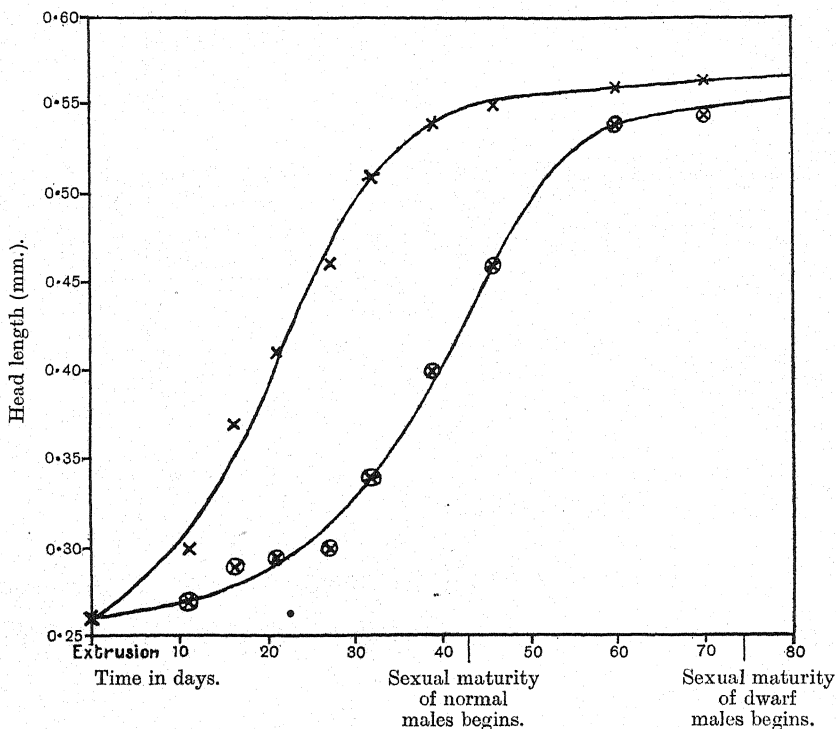
Fig. 1. Growth of normal and dwarf *Gammarus* at 23° C. Averages of head-length. (15 normals; 9 dwarfs.)

In the segregating families the average head-length for the normal and dwarf specimens at different times is given in the following table; see also Fig. 1.

TABLE III.

Days from extrusion	Head-length (in mm.)		Ratio, dwarf/normal %
	Normal	Dwarfs	
0	0·26	0·26	100
10	0·30	0·27	90
16	0·37	0·29	78
21	0·41	0·30	73
27	0·46	0·30	65
32	0·52	0·34	65
46	0·55	0·46	84
60	0·56	0·54	96

It will be noticed that both dwarfs and normals are of the same size at extrusion. During the first 27 days of their development, however, the head-length of the dwarfs only increases by 0·04 mm., compared with 0·20 mm. for normal specimens during the same period. Thus the difference between the two types increases at first (the upward-concave portion of the S-shaped growth curve) and then diminishes again after the point of inflection.

In addition, the growth-rates of dwarf and normal specimens have different temperature-coefficients. Two segregating families have been kept at 14° C. It was then found that the average head-length of the dwarfs is 0·42 mm. at the time when that of the normal specimens is 0·52 mm., whereas at 23° C. it is only 0·34 mm. for the same normal head-length; *i.e.* the dwarf/normal head-length ratio is 81 per cent. instead of 65 per cent. It is at present impossible to give any detailed account or explanation of this phenomenon. It has only recently been detected and further work on it is in progress. A more adequate picture of the great size-difference between normals and dwarfs is obtained by taking the cube of the head-length as standard. Using this as an indication of bulk, we find that the ratio of the bulk of the dwarfs to that of normals of head-length 0·52 mm. is only 53 per cent. at 14° C., 27 per cent. at 23° C.

(4) THE EFFECT OF DWARFING ON THE EYE-COLOUR.

The dwarfs described in this paper all belonged to material carrying the recessive mutations red (**rr**) and no-white (**ww**). That is to say the normal black pigment of the eye is absent, so that the facets are scarlet at birth, and they are not separated by the normal white inter-facetary pigment. It has been shown (Ford and Huxley, 1927) that, at a constant temperature of 23° C., the **rr** eyes gradually darken owing to a deposition of melanin, the rate at which this darkening takes place being controlled genetically.

The facets of normal-sized *Gammarus* of the constitution **rrss** (**s** being a factor for moderately slow melanin deposition) begin to darken at about 4 to 8 days from extrusion, and reach an equilibrium condition (of a chocolate shade) in about 5 to 7 weeks. Those of the constitution **rrSS** (**S**, the allelomorph of **s**, being a factor for rapid melanin deposition) begin to darken at about 4 to 6 days from extrusion and reach an equilibrium condition (of a deep chocolate colour) in about 3 weeks. Only very rarely are they quite black, and they generally become slightly paler as they increase in size. Later, as new facets are

developed, they too appear red at first and pass through a similar colour series.

The dwarfs, on the other hand, both of the rapidly and moderately slowly darkening strains, acquire jet black eyes after about 10 to 12 days; they may become slightly lighter when the facets eventually increase in size. The later formed facets, however, are never jet black, being scarlet at first and darkening until they correspond pretty closely in depth of pigmentation to non-dwarf eyes of the same age. At certain stages, therefore, the area first formed appears as a blackish patch in the middle of newly formed red or brownish facets.
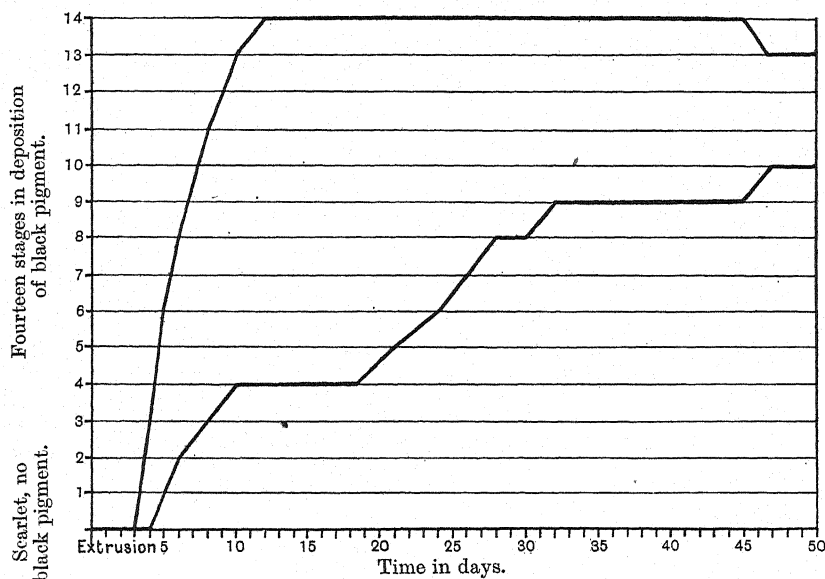
Fig. 2. Average darkening of original (central) facets in the eyes of normal and dwarf *Gammarus chevreuxi* at 23° C. (Normals, lower line, 20 specimens; dwarfs, upper line, 9 specimens.)

Very few dwarf specimens of the constitution **rrSS** have been examined. They behave very like the **rrss** animals, though the darkening appears to be a little more rapid; the eyes seem to become black in about 8 to 10 days. Fig. 2 illustrates the average darkening of dwarf and normal *Gammarus* of the constitution **rrss** in standard conditions of temperature (23° C.) and food.

The phenomenon of rapid darkening of the facets is to be noticed in all dwarfs, whether they are of a genotypic or phenotypic nature.

Phenotypic dwarfing, however, is much more variable in degree, time of onset, etc., and its effects on the eye-colour are thus less clear-cut.

(5) Discussion.

All observations bearing upon the interrelation of Mendelian factors with development are of interest. What appears to be a somewhat new type of such interrelation has been recorded from *Gammarus chevreuxi*, and there is some indication that it may throw light on the physiology of inheritance in other groups, such as Insects and Mammals (Ford and Huxley, 1927).

In the first place, it has been shown that at least four pairs of genes, which determine different shades of eye-colour between red and black, do so by altering the rate at which melanin is deposited in the originally red eye. The rate is usually correlated with the time of onset of pigmentation and with the final density attained, though in one case the action is concerned solely with the time of onset (Ford and Huxley, *l.c.*).

In the second place, it has been shown that in the unfertilised egg, under the influence of the maternal genes, there is normally formed a precursor for the red pigment of the eye. When, as in *white-body*, no maternal gene for red pigment exists, the precursor is not formed, and therefore if the red-determining gene is introduced from the father, the eye is at first colourless (white), several days having to elapse before the red precursor can be formed and turned into red pigment (Sexton and Pantin, 1927).

The third case concerns the interrelation between moderate rates of melanin deposition and dwarfism (slow growth) described in Section 4 of the present paper, and the facts there brought forward call for some explanation. The following suggestion, which would account for the observed phenomena, has been made to me by Prof. J. S. Huxley.

The black eyes of the young dwarfs would at first suggest that the melanin is formed in the same absolute amount as in non-dwarfs, irrespective of the size of the body; accordingly, since the dwarf's eye is so much smaller than normal, the melanin has to be much more concentrated, and the eye appears black. On the other hand, the lighter colour of the later facets does not support this interpretation.

It may plausibly be suggested that in the egg of the dwarfs (which is of normal size since the dwarf is merely a slow-growing form, and does not reproduce until it attains approximately the normal reproductive size of the species) a precursor for melanin pigment is formed, of course in proportion to the size of the egg—*i.e.* in normal amount. This, after

the normal time, is turned into melanin. But meanwhile the animal's eye-area has only grown to less than half that attained by the normal in the same time. The amount of melanin is therefore relatively much too great, and the eye at first appears black. Later supplies of melanin, however, will be produced in relation to the size of the animal, and accordingly later facets will not be abnormally dark (though there may be a slight excess darkening due to lag).

It is hoped to test this hypothesis by means of reciprocal crosses of rapid-darkening red with albino dwarfs.

(6) SUMMARY.

1. Dwarfing in *Gammarus* may be either of environmental or genetic origin.

2. Environmental dwarfing may be produced by bacterial infection, improper food, and probably in a number of other ways.

3. Genetic dwarfing has been shown to depend upon a single recessive factor, which has been called **g** (slow growth; as against **G**, normal growth). This reduces growth-rate, but not size at maturity or final size.

4. Experiments on dwarfing, as on eye-colour, must be conducted at a constant temperature (23° C. in the present case). Not only do *Gammarus* grow at different rates at different temperatures, but the relative growth-rate of the dwarfs to that of the normals also varies, *i.e.* the growth-rates of dwarfs and normals have different temperature coefficients.

5. Dwarfing has a marked effect upon the eye-colour. The eyes of dwarfs carrying the **rr** factor-pair for red facets rapidly darken to a jet black, instead of darkening slowly to a chocolate shade. However, new facets subsequently added darken at the normal rate, and never become black.

6. The eggs of the dwarfs, since they are merely slow-growing forms, are of normal size. It is suggested that a melanin-precursor is formed in the eggs; this will be of normal amount. Since growth is retarded, however, the melanin thus formed will have to be deposited in an eye much smaller than normal, which will therefore appear dark. Further production of melanin, however, will be in proportion to body-size, and the later facets will therefore be paler.

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ON THE CYTOLOGY OF SPELTOID WHEATS IN RELATION TO THEIR ORIGIN AND GENETIC BEHAVIOUR.

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(With Thirty-eight Text-figures.)

EXTENSIVE genetical studies of the speltoid forms of wheat have been made by a number of workers, who have advanced various divergent theories to account for the origin of these forms and for the peculiar segregation ratios obtained from different strains.

The fact that forms closely resembling *T. Spelta* L. arise in pure lines and varietal crosses of *T. vulgare* Host., and that some speltoids give rise to forms resembling *T. compactum* Host. in density of ear, is of great interest from the systematic point of view, and the elucidation of the problem may be expected to throw considerable light on the phylogeny of cultivated wheats. The chief genetic complexity arises from the fact that phenotypically similar heterozygous speltoids may give very different progeny ratios, and that these are evidently determined by some form of heterogamy and by zygotic elimination.

Kajanus (1927, pp. 217–229) has summarised the literature of this problem and given an extensive bibliography, so that only points of special application need be reviewed here.

Different workers have grouped their speltoid strains under three, four or more genetic types, although there has been considerable deviation between different strains within each type. Nilsson-Ehle groups his numerous strains into three types which he designates A, B and C, and for the present this classification is, with certain reservations, here adopted. (A) type heterozygous speltoids give normal type, heterozygous speltoid and homozygous speltoid progeny in ratios approaching 1 : 2 : 1, but with varying degrees of deficiency in the latter classes. (B) type heterozygotes give about four or five times as many heterozygous as normal segregates, and only a very few homozygous speltoids, which are more or less weak and sterile. (C) type heterozygotes usually give more normal than heterozygous speltoid progeny, and again only a few weak and sterile homozygous speltoids. The (B) type occasionally arises from the (C) type.

Winge (1924) described the cytological conditions in two strains of speltoids, and in five other related aberrant types of wheat. The first speltoid strain examined was obtained from Åkerman, and the remainder from Lindhard. From 72 grains of Åkerman's strain, Winge obtained 25 normal type plants, 15 heterozygous speltoids, and 3 homozygous speltoids, so that it was evidently of type (C). Winge made a cytological examination of the heterozygotes, and found them to have the normal number of chromosomes, viz. 42. These divided regularly, 21 going to each pole in the first reduction division of the pollen mother cells. The strain of speltoids from Lindhard's material was of the 1 : 2 : 1 type. Heterozygous speltoids of this strain were found to have the normal number of chromosomes, but frequently an unpaired chromosome was observed, and a trivalent was seen in a few cells. Homozygous speltoids of this strain were also found to have 42 chromosomes, but large complexes, presumably consisting of four chromosomes, were observed in some of the pollen mother cells. A "Square-head heterozygote" and a *perennis* type were each found to have only 41 chromosomes. A *compactum* heterozygote and a dwarf club type, however, appeared to be cytologically normal. A dwarf *compactum* type had abnormal anthers and degenerated pollen mother cells.

Lindhard, without cytological evidence, had earlier suggested that speltoids and the related aberrant forms probably arise through chromosome irregularities. Winge developed this hypothesis and gave formulae to represent the chromosome constitution of the types examined, and also of other types which he assumed should exist. The hypothesis is based upon the fact that cultivated wheat, *T. vulgare*, is phylogenetically a hexaploid species, and upon the assumption that its 21 gametic chromosomes consist of three more or less similar sets of 7, and that, on account of the similarities, faulty conjugations sometimes occur. The triplicated set of chromosomes assumed to carry the factors determining the head characters of wheat under consideration are represented by Winge as $\frac{ABC}{ABC}$. Normally A must pair only with A, B with B, and C with C, but a faulty conjugation of $A + A$, $B + C$, $C + B$ could give a gamete ABB, which when paired with a normal gamete would give the zygote $\frac{ABB}{ABC}$, which formula Winge takes to represent a heterozygous speltoid. By postulating that in different strains there are varying degrees of affinity between these chromosomes with consequent differences in their segregation at meiosis, and by assuming differential viability of aberrant male and female gametes, Winge attempted to show that

speltoids of the formula $\frac{ABB}{ABC}$ could produce various progeny ratios. He further postulated that heterozygous speltoids with the formula $\frac{ABo}{ABB}$, and "homozygous" speltoids $\frac{ABo}{ABB}$ and $\frac{ABBB}{ABB}$ could occur, but was unable to confirm their existence cytologically.

Following Winge's work, a cytological study of speltoids from Nilsson-Ehle's material was undertaken by Ekstrand, but only normal numbers and conditions were found in the material examined (Nilsson-Ehle, 1927).

In the course of a genetical and cytological study of fatuoid oats (Huskins, 1927, and unpublished data) different ratio-types of fatuoids were obtained which were very closely analogous to the ratio-types of speltoids mentioned above, and each of these types was found to have a distinctive chromosome number and behaviour. These facts were explained on a hypothesis essentially similar to that of Winge, though with certain significant modifications. In order to discover whether the analogy between fatuoids and speltoids extended to their chromosome constitution, a study of speltoids was begun on strains kindly supplied by Professor Nilsson-Ehle and Dr Å. Åkerman. Particulars of the genetic behaviour of some of these strains were supplied, but not of others, so that the cytological examination should be as unbiassed as possible. For the same reason the cytological observations are now being described before progeny tests are made. Seeds have been saved from all plants from which cytological material was taken, and the tests will be made in 1928. These are particularly necessary in view of the sudden changes in ratio-type which sometimes occur in speltoids. The cytological study alone seems to have cleared up many of the difficulties, but extensive discussion of the speltoid problem will be deferred until the genetic evidence is also available.

METHODS.

Preliminary cytological fixations were made from greenhouse plants during the winter of 1926–7 with Allen's Bouin, Carnoy, Flemming and "Kihara" (1924) fixatives, and various modifications of the latter. Allen's Bouin gave the most uniform results, and a high general standard of fixation, but Kihara's method, while somewhat variable, gave results which were in most cases superior to any of the others. This method consists in fixing for one or two minutes in Carnoy (6 : 3 : 1) and then for 24 hours in Flemming. In a modification of it which gave similar results, Zenker fluid with only 1 or 2 per cent. of acetic acid was substituted for

Flemming. This obviated the necessity for bleaching. Fixations of field-grown material in 1927 were made principally by the Carnoy-Flemming or Carnoy-Zenker method. The anthers were dissected out in some cases, but fixations were chiefly made of spikelets with the ends of the glumes clipped off.

Newton's iodine-gentian-violet staining method (Huskins, 1927) was used almost exclusively, and gave results far superior to any other. It gives a particularly brilliant stain after the Carnoy-Zenker fixative.

One hundred and eleven different fixations were made from a total of 83 plants, but these have not quite all been examined and it was not possible to determine the cytological conditions satisfactorily in all plants studied. All material was embedded in paraffin and was cut mainly at 14μ. The study was almost entirely confined to the pollen mother cells, but a few examinations have been made of embryo-sac mother cells and of root tips, the latter fixed in medium Flemming and Benda.

Speltoids of Type (A).

One heterozygous speltoid plant newly arisen in an F_9 family of Extrakolben × Brown Schlanstedt was given to me by Dr Åkerman, and numbered 26–74. In 1927 it gave 14 normal type, 21 heterozygous and 9 homozygous speltoid progeny, and so is of the (A) type. One plant of each class was examined cytologically. All have 42 chromosomes. No deviation from the normal arrangement of 21 bivalents (21_{II}) was found in the normal plant. A few divisions were somewhat irregular, but no features of significance could be determined. The heterozygous speltoid plant showed a trivalent and a univalent in many of its pollen mother cells, though, naturally, it was not always possible to prove the existence of both in the same cell. The trivalents were all of the end-to-end chain type (Figs. 1 and 2). The homozygous speltoid plant showed a quadrivalent (Figs. 3 and 4) in many of its pollen mother cells, and a trivalent was seen in two of them.

One other strain of speltoids, given to me by Dr Åkerman under the number 1924–443, was found to be cytologically similar to the above strain 26–74, except that the trivalents in the heterozygotes, and the quadrivalents in the homozygous speltoids, seemed to occur rather less frequently, and the trivalents were not always of the end-to-end chain type. No information about this strain was given except that it had been a constant type for many years. The genetic evidence from the limited number of plants grown in 1927 clearly shows it to be of the (A) type, but in some cases I have not been able to determine with
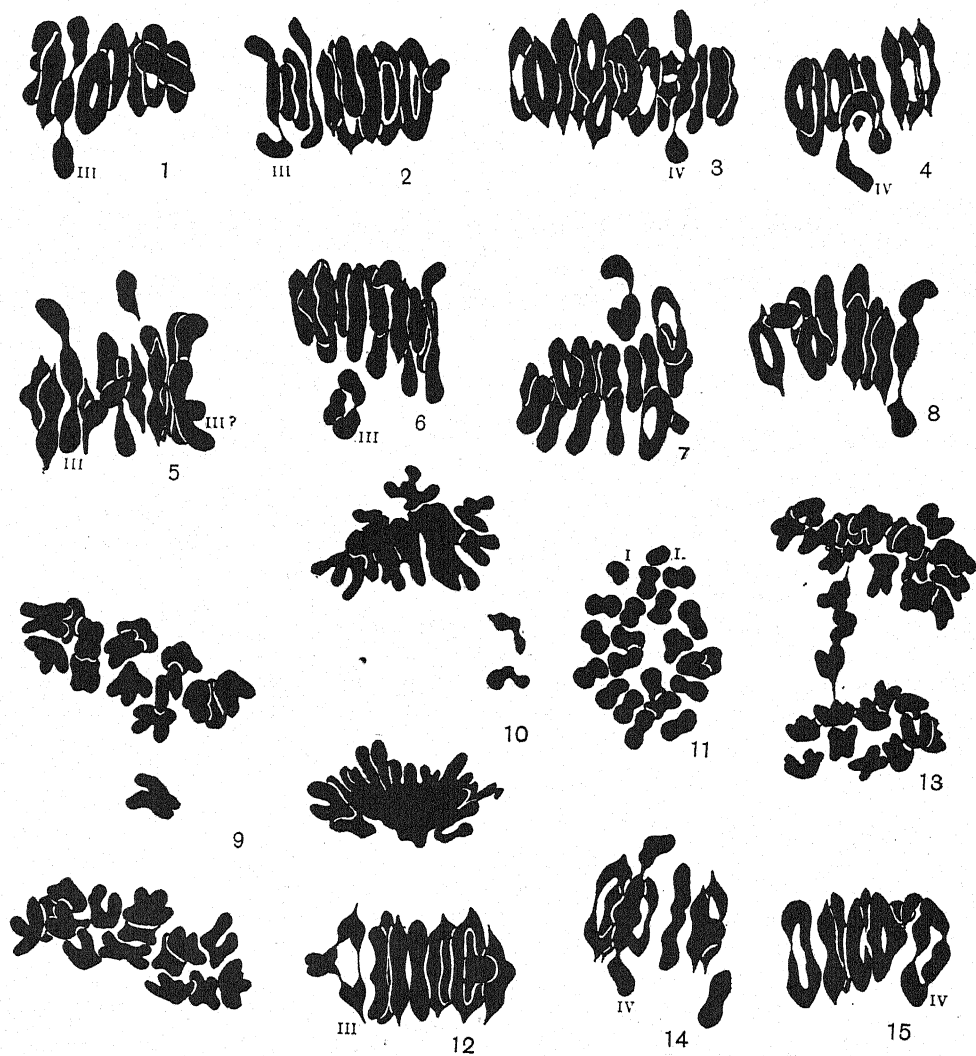
Figs. 1–15, heterotypic divisions of type (A) speltoids. See pp. 106 and 108. *ca.* 2200 ×.

certainty whether segregates were heterozygous speltoids, or beardless homozygous speltoids. Two normal type plants of this strain were cytologically examined. One showed only the normal arrangement of 21_{II}, but in the other, out of a total of about 250 cells examined there was one cell showing both a trivalent and a possible trivalent (Fig. 5). Three heterozygous speltoid plants all showed a trivalent in a fair proportion of their cells. These trivalents were of various shapes (Figs. 6, 7 and 8).

It should be mentioned that difficulty is occasionally experienced in distinguishing between trivalents of the shape shown in Figs. 12, 13, 23, etc., and metaphase bivalents that result from the "key-ring" type of union. Where any doubt is felt, the structures will here be termed possible or probable trivalents, and the doubt indicated in the figures by a question mark.

A univalent was seen splitting on the plate during the late anaphase and telophase (Figs. 9 and 10) in a number of cells. Occasionally in polar views of the metaphase of the first division a loosely united bivalent was seen, or two univalents (Fig. 11). Three plants of rather doubtful classification, being either heterozygous speltoids or beardless homozygotes, though probably the former, all showed trivalents in many of their cells, several of these in one plant, No. 26–59$_2$, being of a shape not seen in other plants of this strain (Figs. 12 and 13), but similar to those in strain 1924–444 to be described later. A possible quadrivalent was seen in one pollen mother cell of one of these doubtful plants. One homozygous speltoid plant was examined and it showed quadrivalents in a fair number of its pollen mother cells (Figs. 14 and 15).

Speltoids of Type (B).

Satisfactory fixations were obtained of two normal type and six heterozygous speltoid plants from a strain of (B) type speltoids supplied by Professor Nilsson-Ehle as No. 26–1292. The normal plants have the normal chromosome number and normal divisions. Each of the hetero-zygotes has only 41 chromosomes. In almost every case these form $20_{II} + 1_I$. The bivalents separate normally, 20 going to each pole in the first division, while the univalent splits longitudinally on the plate in the late anaphase, similarly to that in Figs. 9 and 10. Very fre-quently the halves of this univalent arrive at the poles too late to be included in the daughter nuclei, Fig. 16. Good counts of $20_{II} + 1_I$ were obtained in many polar-view metaphase plates (Fig. 17), and of 20 chromosomes plus one split univalent in each of the late
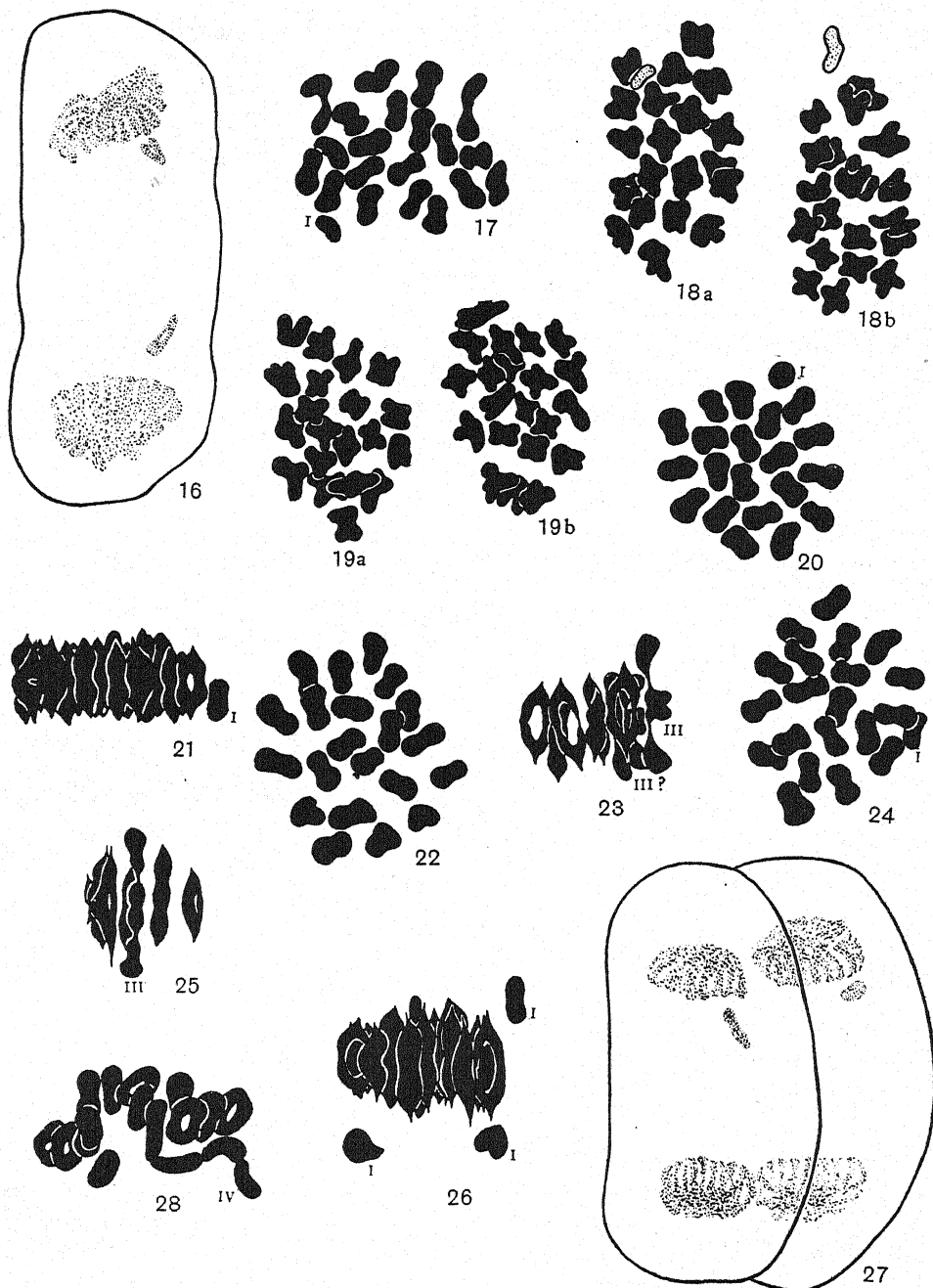
Speltoids of type (B). Figs. 16–26, heterotypic divisions. Fig. 27, homotypic division. Fig. 28, heterotypic metaphase of an abnormal plant from type (B), showing quadrivalent. See pp. 108 and 110. *ca.* 2200 ×.

anaphase plates (Figs. 18 *a* and *b*). In the second division the half-univalents go at random to either pole as in Fig. 27 and frequently get left out of the tetrad nuclei, as in Fig. 37. In rare cases a separation of 20 and 21 occurs in the first division (Figs. 19 *a* and *b*). No homozygous speltoids were obtained from the 150 seeds of this strain sown in 1927.

Four heterozygous speltoid plants from Åkerman's strain No. 1924–921 were also found to have only 41 chromosomes, good counts being obtained in many pollen mother cells, Fig. 20 ($20_{II} + 1_I$), and also in an embryo-sac mother cell. Again, in practically every cell examined the univalent was found splitting on the plate during the first anaphase, and as in strain 26–1292 the half-univalents are very frequently left out of the daughter nuclei. At the first metaphase the univalent is frequently seen lying at the side of the plate, Fig. 21, or just off it towards one of the poles. Two normal type plants of this strain were found to have the normal chromosome number, Fig. 22. About 350 pollen mother cells of each of these plants were examined at the first metaphase and a trivalent was found in one cell of one plant, and both a trivalent and a probable trivalent in one cell of the other, Fig. 23. In all the other cells the arrangement appeared to be 21_{II}.

Åkerman has described the genetics of this strain in a recent paper (1927). It differs from the ordinary (B) type strains in having produced some moderately vigorous and fertile homozygous speltoid progeny, instead of only sterile, dwarf ones. From 30 seeds of a descendant homozygous speltoid plant sown in November 1926, 10 homozygous speltoid progeny were obtained. One of these was examined cytologically and found to have only 41 chromosomes (Fig. 24). The behaviour of these was different, however, from that found in the 41-chromosome heterozygotes of this and the preceding strain. The odd chromosome was seen dividing on the plate during the anaphase in only about 75 per cent. of the cells examined. A trivalent instead of a univalent was seen in a very large number of cells. These trivalents were of various shapes, but that of Fig. 25 was by far the most common. In no case was a trivalent found to be accompanied by one univalent in this plant. In one cell there were three univalents instead of a trivalent (Fig. 26). Split univalents going at random to either pole were seen in many second divisions (Fig. 27).

Seeds of two heterozygous speltoids from his strain No. 1924–440 were given to me by Dr Åkerman as a 1 : 5 or (B) type strain. The progeny of these two plants were, however, found to be very different. Forty

seeds from his "plant 4" (here numbered 26–55) gave 8 normal type and 26 heterozygous speltoid progeny. One normal and three heterozygous plants have been cytologically examined. The normal has the normal chromosome number and behaviour, and the three heterozygotes each have 41 chromosomes arranged regularly as $20_{II} + 1_{I}$. The cytology and genetics of the progeny of this one plant are therefore in accord with those of the other two (B) type strains.

The progeny of "plant 5" (numbered 26–54) were all much more vigorous than those of its sib, and they were very difficult to classify. Some of them are much closer in appearance to *T. Spelta* than any other speltoids I have seen. Three heterozygous speltoid, 2 probable homozygous speltoid, and 2 sub-compactum plants have so far been cytologically examined, and all found to have the normal chromosome number. The arrangement of the chromosomes, however, is exceedingly irregular. In the heterozygotes polar-view metaphase plates of the following arrangements have been found: 21_{II}, $20_{II} + 2_{I}$, $19_{II} + 4_{I}$, and $19_{II} + 1_{III} + 1_{I}$. In the probable homozygous speltoid plants a quadrivalent (Fig. 28) or a trivalent occurs very commonly, and the following arrangements have been found: 21_{II}, $20_{II} + 2_{I}$, $19_{II} + 1_{IV}$, and $18_{II} + 1_{III} + 3_{I}$. One cell was found with two trivalents, in side view. In the two sub-compactum plants good plates of 21_{II} were found, but these plants have not yet been studied extensively.

It is obvious both from the genetical and cytological evidence that some change has occurred causing this plant to deviate from the (B) type. A complete progeny test will be made in 1928, and pending the results of this, further cytological study does not seem warranted. It may be that "plant 5" resulted from a natural cross, in which case the male parent was probably *T. Spelta*, or it may have arisen by further chromosome aberration. Perhaps Dr Åkerman will be able to throw light on this question when he has completed the analysis of his genetic results with this strain, which he informs me he is now preparing for publication.

Speltoids of Type (C).

The (C) type is defined by Nilsson-Ehle (1921) as one in which the heterozygous speltoids produce more normals than heterozygous speltoids, and very few homozygous speltoid progeny. As shown in his tables 10, 11 and 12, however, this definition does not always hold. Within a single family the ratio of normals to heterozygotes varies from 2 : 1 to 1 : 2, and in certain cases (1921, p. 68) he experiences difficulty in classifying

strains. It would seem that the definition of the types should be amplified. Probably more stress should be laid upon the relative vigour of the homozygous speltoid segregates, which are weaker in the (B) and (C) types than in the (A) type.

Fifty seeds from each of three heterozygous speltoid plants of Nilsson-Ehle's "(C) series strain 26–1295" gave 48 normal type, 67 heterozygous speltoid and no homozygous speltoid progeny in 1927, a total of only 115 plants from the 150 seeds. Four heterozygous speltoid and two normal type plants were examined cytologically. The four heterozygous plants were all found to have 43 chromosomes. At the first metaphase these are probably usually arranged as $20_{II} + 1_{III}$, but owing to the irregularity of the divisions it is not always possible to determine this with certainty as in Fig. 29. In side views of the first metaphase the trivalent is seen fairly often, the usual shape being an end-to-end chain, similar to that in Figs. 1, 2, etc., but sometimes of the type shown in Fig. 23.

It must be mentioned that both in fatuoids and speltoids the divisions have generally been found to be much more irregular in plants with 40, 43, or 44 chromosomes, than in those with 41 or 42. The irregularities frequently make it very difficult to determine the chromosome number with certainty.

Occasionally the 43 chromosomes of these heterozygous speltoids are arranged as $21_{II} + 1_{I}$ (Fig. 30) or very rarely as $20_{II} + 3_{I}$.

In one of these four heterozygous speltoids there is a further cytological abnormality in the loss of approximately half a chromosome. This probably has no bearing on the speltoid problem, as the trivalent which is characteristic of these plants can in some cases be seen in the same cell as the bivalent with one member only half the size of the other. This case of "deficiency" will be described in a separate paper, as it has several points of general cytological interest.

One of the normal type plants examined has the normal chromosome number and regular behaviour as expected, but the other plant, No. 26–95$_\frac{1}{3}$, has only 41 chromosomes. A univalent occurs in it very frequently and a trivalent very occasionally. In the trivalent shown in Fig. 31 it will be noted that two of the members have distinct subterminal constrictions. The inner end of the left hand chromosome is turned slightly downwards so that one cannot determine with certainty whether it has a constriction or not. Such constricted chromosomes have never been observed in trivalents occurring in speltoid plants. The progeny test of this plant may be expected to yield interesting results.
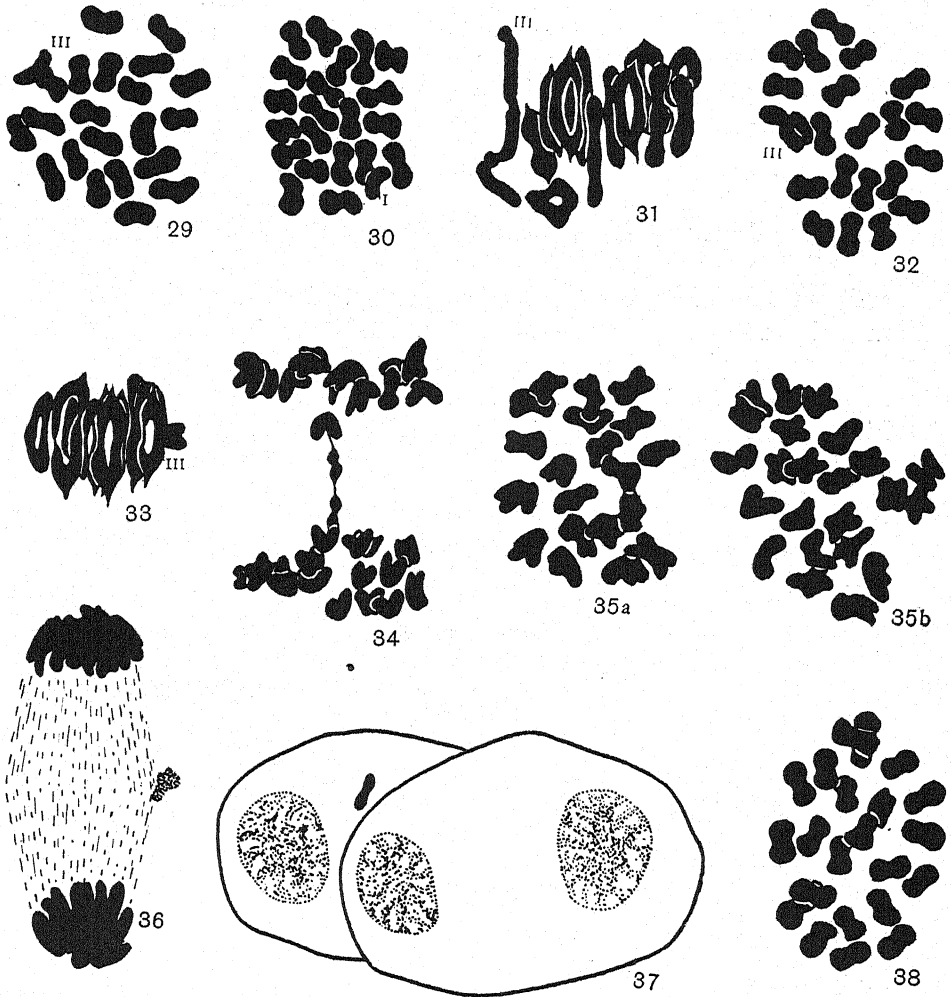
Speltoids of type (C). Figs. 29–35 and 38, heterotypic divisions. Figs. 36 and 37, homotypic divisions. See pp. 112 and 114. *ca.* 2200 ×.

Seeds of his speltoid strain 1924–444 r 27 were supplied by Dr Åkerman without information regarding its genetic nature. One hundred and thirty seeds from three heterozygous speltoid plants gave 40 normal type, 48 heterozygous and 2 homozygous speltoid progeny. Although the heterozygotes are here again slightly more numerous than the normals, as in Nilsson-Ehle's (C) series strain 26–1295, this strain is also here classed as (C) type. In a recent letter, Dr Åkerman informs me that he has obtained from it approximately equal numbers of heterozygotes and normals, and only 1–4 per cent. of homozygous speltoids, and that he therefore classifies it as an (A) type strain. The difference is clearly one of definition only, and does not affect the conclusions.

Satisfactorily fixed material was obtained from two of the normal and four of the heterozygous speltoid progeny. The normals have the normal chromosome number and fairly regular behaviour. All four heterozygous plants were found to have 43 chromosomes. Counts of $20_{II} + 1_{III}$ were obtained in many cells at the first metaphase, as in Fig. 32. The trivalent is here of the type which is illustrated in side view in Fig. 33. This type of trivalent was found to occur much more frequently than the chain type in this strain. Probably in conformity with this fact is the observation that the trivalent frequently divides into two equal halves in the first division, *i.e.* the centre number splits longitudinally, as shown in Fig. 34. In polar views of a number of cells at the first anaphase, each plate was found to contain 20 normal size chromosomes and one large one irregular in shape, which was presumably a half trivalent. One cell was found with 20 chromosomes in one anaphase plate, and 23 in the other (Figs. 35 *a* and *b*). In the latter, three of the chromosomes are attached to each other. In the second divisions a chromosome disintegrating in the cytoplasm (Fig. 36) or being left out of the tetrad nuclei (Fig. 37) was seen fairly frequently.

Sixty seeds from two homozygous speltoid plants produced 27 homozygous speltoid progeny, all rather weak and more or less sterile. Three of these plants were examined. All were cytologically very irregular, and the chromosome number could not be determined with certainty from pollen mother cell divisions of two of them, though they probably have 44 chromosomes each. Root-tip counts tend to confirm this in one of them, but it is very difficult to get somatic chromosome counts for wheat with an accuracy greater than ± 1. In the third plant the chromosome number is clearly 44 (Fig. 38).

DISCUSSION.

In this discussion it is proposed to refer mainly to the cytological observations and their bearing on some of the most important genetic features of speltoids. The general considerations which underlie the hypothesis presented have been discussed in a recent paper on fatuoids (1927) and detailed genetic considerations of speltoids will be left to a later paper.

From the evidence presented it seems certain that the speltoid strains under consideration have arisen from *T. vulgare* through chromosome aberration. The fact that such forms can also arise through certain interspecific crosses bears only on the general phylogenetic considerations involved, and so need not be considered further in relation to the immediate problem.

It also seems clear that the differences between the principal ratio types are determined primarily by differences in chromosome number. The only possible exception to this rule yet found is in the progeny of plant 5 of strain 1924–440 (cf. p. 111), and it need scarcely be considered further until genetic evidence is available from the plants which have been cytologically examined.

Winge assumed that different affinities between the B and C chromosomes in different strains might cause heterozygous speltoids of the formula $\frac{ABB}{ABC}$ to give widely different progeny ratios. This assumption now appears to be unnecessary although it may still serve to explain some of the minor genetic variations within each type. The different chromosome numbers found in (B) and (C) type speltoids render necessary an extension in Winge's scheme, but his formula $\frac{ABB}{ABC}$ may be accepted for the present as representing a heterozygous speltoid of type (A). The chromosome complement of $19_{II} + 1_{III} + 1_{I}$ frequently found in these plants is in accord with this formula, the trivalent presumably being composed of the three B chromosomes and the univalent being the odd chromosome. The fact that the complement is more frequently 21_{II} in which one of the B chromosomes has presumably mated with the C chromosome may be attributed to the complicated relationships which must exist between the different chromosomes owing to the hexaploid nature of wheat.

The commonest type of homozygous speltoid from an $\frac{ABB}{ABC}$ heterozygote would be expected to have the formula $\frac{ABB}{ABB}$. The complement of

$19_{II} + 1_{IV}$ frequently found in these plants is in accord with the formula, but again it is not found regularly, as metaphase plates of 21_{II} commonly occur.

If a heterozygous speltoid of the formula $\frac{ABB}{ABC}$ regularly formed 21_{II}, it would be expected to give normal type, heterozygous speltoid, and homozygous speltoid progeny, represented respectively by $\frac{ABC}{ABC}$, $\frac{ABB}{ABC}$, and $\frac{ABB}{ABB}$, in a ratio of 1 : 2 : 1. The fact that the genetic ratios of type (A) speltoids sometimes vary greatly from this may in turn be attributed in large part to the variations which occur in chromosome pairing. While such an interrelated explanation of both the cytological and the genetical variation is not entirely satisfactory, it does appear probable that a causal relationship exists between them. Further, it seems significant that the trivalents and quadrivalents appear to occur more frequently in the speltoid segregates of the newly originated strain than in the segregates from the (A) type strain of longer standing. The same observation was made in fatuoids.

Continuing on the assumption that the characters of wheat under consideration are determined by the interaction of factors in the chromosomes designated B and C and that any excess of B chromosomes over C chromosomes produces a speltoid, we may now represent the 41-chromosome heterozygous speltoids of the (B) type by the formula $\frac{ABo}{ABC}$. The regular occurrence of a univalent agrees well with the expectation from the formula $\frac{ABo}{ABC}$. Since the split halves of the univalent are very frequently left out of the daughter nuclei, as here shown in the pollen mother cells, it follows that many more male gametes with 20 chromosomes are formed than with 21, and Watkins (1925) has shown that in interspecific hybrids of wheat there is a slightly greater tendency for the univalents to be left out of the daughter nuclei in embryo-sac mother-cell divisions than in pollen mother cells. The proportion of 20 and 21-chromosome gametes formed is almost undoubtedly affected by various environmental factors, and this is probably the primary cause of the wide fluctuations in the proportion of normals to heterozygotes that occur in strains of type (B). Gametes with only 20 chromosomes, especially male ones, may be expected to function less frequently than those with the normal number (Watkins, 1925, and others) and plants with only 41 chromosomes have been shown to be weaker than those with 42 (Huskins, 1927). Differences in chromosome number are, therefore, undoubtedly the principal cause of the heterogamy and

differential zygotic elimination which in turn affect the genetic ratios.

Without further detailed genetic evidence than that yet published it is impossible to formulate any scheme that will give more than a rough idea of the mode of segregation of unbalanced speltoid types. Studies of the degree of heterogamy have been made by Nilsson-Ehle, Lindhard, and Åkerman, but in a recent paper Nilsson-Ehle (1927) states that some of the earlier conclusions were incorrect. He promises further results shortly. Lindhard has especially studied the zygotic elimination also, but experiments are still required on different strains. In certain wheat hybrids Watkins (1925) has demonstrated that male gametes with aberrant numbers function much less frequently than those with the normal chromosome complement, but that there is very little if any selective elimination of female gametes. Kihara (1924), however, stresses the importance of the zygotic elimination of plants with unbalanced chromosome numbers in his studies of wheat hybrids. The necessity for further information about both gametic and zygotic elimination in speltoids is therefore evident.

From the cytological evidence it is at least clear, however, that a heterozygous speltoid of the formula $\frac{ABo}{ABC}$ should produce many more progeny like itself than normal type progeny with the full chromosome number.

Homozygous speltoids of the (B) type would be represented by the formula $\frac{ABo}{ABo}$ and have only 40 chromosomes, which would account for their weakness and sterility. Unfortunately none were obtained in the 1927 sowings, but 40 has been found to be the characteristic chromosome number of the analogous homozygous fatuoids. From fatuoid studies there is also evidence that zygotic elimination, both in the embryonic stage and during the growing season, is a very large factor in limiting the number of 40-chromosome progeny.

Either by natural crossing with a speltoid of the (A) type or by chromosome aberration, a (B) type heterozygous speltoid could on occasion give rise to a "homozygous" speltoid of the constitution $\frac{ABo}{ABB}$, and this formula may be taken to represent the more or less vigorous and fertile "homozygous" speltoid from Åkerman's strain 1924–921. The fact that a trivalent is formed very frequently in this plant indicates the validity of a formula containing three B's. The term "homozygous speltoid" is of course applied to forms like $\frac{ABo}{ABB}$ only in a descriptive

sense, to indicate their phenotype in conformity with the terminology of previous workers.

The 43-chromosome heterozygous speltoids of type (C) may now be represented by the formula $\frac{ABCB}{ABC}$. The fairly regular occurrence of a trivalent in these plants agrees well with the formula. Just as (B) type heterozygous speltoids form a larger number of 20-chromosome than of 21-chromosome gametes, so the 43-chromosome heterozygotes of type (C) form more gametes with 21 than with 22-chromosomes. There is evidence from fatuoids that 22-chromosome gametes function more frequently than 20-chromosome gametes and that 43 and 44-chromosome plants are more viable than 41 and 40-chromosome plants. Heterogamy and differential zygotic elimination may therefore be expected to affect the progeny ratios less in (C) type speltoids than in the (B) type strains, but the situation is undoubtedly similar.

The fact that one of the homozygous speltoid progeny of a homozygous speltoid segregate from Åkerman's strain 1924–921 has 44 chromosomes and that this complement very frequently includes a quadrivalent, indicates the validity of the formula $\frac{ABCB}{ABCB}$ for this class of segregate, though it would of course be more satisfactory to have counts from the immediate offspring of a heterozygote.

The occasional origin of (B) type speltoids from the (C) type and the extreme rarity of the reverse change are also explicable on the cytological evidence. The irregular divisions frequently seen in 43-chromosome plants could produce occasional gametes of the formula ABo, and these mated with normal gametes give (B) type heterozygotes. On the other hand the production of an $ABCB$ gamete by a 41-chromosome plant would occur extremely rarely, if ever.

The principal genetic differences between heterozygous speltoids of the different ratio-types are therefore seen to be explicable on the basis of differences in chromosome number. The differences in vigour between the different classes of segregates within each type, and the differences between similar segregates of the different types, are also accounted for on the same basis. As in fatuoids, it appears that a difference of ± 1 chromosome from the normal number makes only a slight difference in vigour and fertility, but that a difference of two chromosomes causes the plant to be more or less dwarf and sterile. An excess of two chromosomes evidently has less effect than a deficiency of two.

It seems probable, however, that the formulae in the scheme here submitted may have to be modified as further evidence becomes

available from other strains. It may well be, for instance, that the chromosomes designated A carry certain factors similar to those in the C chromosomes, as was suggested in the case of fatuoids. If this should be so the scheme may be expanded considerably without any significant changes being necessary.

In the plants so far studied cytologically it has been necessary to consider only whole chromosome differences. Deficiencies similar to that found in one plant of strain 26–1295, but in chromosome A, B, or C, and possible cross-overs between B and C, would, however, produce many complications. It may be that such changes as these will be found to be responsible for some of the minor genetical peculiarities of speltoids and for the occurrence of "part-mutations" such as those described by Nilsson-Ehle (1927).

Possible relationships of the other aberrant forms which arise in the progeny of some speltoid strains have been mentioned by Winge, but cannot be further discussed with profit until more details of their cytology are available. It is interesting however to note that the "Square-head heterozygotes" found by Winge to have only 41 chromosomes give Square-head plants and heterozygous speltoids in a ratio of 5 : 1. The situation is here exactly the reverse of that in (B) type speltoid heterozygotes and gives an indication of the possibilities. It is extremely likely that the most regular genetic features of these other aberrant forms of wheat are also determined by chromosome aberrations similar to those here described, though the situation is undoubtedly complicated by natural crossing which occurs very much more commonly in cytologically unbalanced plants than in normal wheat or oats.

The occurrence of 20–23-chromosome segregation in (C) type heterozygotes, illustrated in Figs. 35 a, b, would be expected to produce plants lacking a B chromosome. The three united chromosomes are presumably the three B's, and the 20-chromosome gametes which would result from divisions of this type would therefore be represented by AoC. Such a gamete mated with a normal one would give a zygote $\frac{AoC}{ABC}$, which, according to Winge, would be a compactum heterozygote.

Until genetic results are available from the 41-chromosome normal type plant of strain 26–1295 it is scarcely profitable to speculate regarding its constitution and formula.

On account of the possibility of trivalents being formed by chromosomes other than the ones assumed to carry the speltoid factors, these wheat plants are unsuitable material for close cytological studies of the significance of trivalent shape. Where a somewhat unusual type of

trivalent occurs more or less consistently, however, as in strain 1924–444, the possibilities may be considered of a segmental interchange producing a chromosome with similar ends, or of the loss of a small piece from one end of a chromosome. If different shapes favour different methods of division, as they appear to do, they may be expected to give slightly different genetic results owing to different possibilities for the omission of chromosomes from the daughter nuclei. One would expect, for instance, that a chain type trivalent would most frequently divide 2–1 at the first division, that all its members would split equationally in the second division, and that very rarely would any be left out of the daughter nuclei. A centrally attached trivalent, on the other hand, might frequently divide into equal parts at the first division, and then the split halves would probably behave similarly to the split univalents of (B) type heterozygotes in the second division, and therefore frequently be left out of the daughter nuclei. More favourable material than hexaploid wheat is necessary for the solution of some of these questions, but it is hoped, nevertheless, that in future studies it may be possible to determine the relationship between some of the minor cytological and genetic differences of different strains.

It is obvious on *a priori* grounds, as well as from the occasional irregularities here reported in normal type plants, that chromosome aberrations may occur in the triplicated sets of chromosomes other than that presumed to carry the factors which determine the characters of wheat at present under consideration. In many cases the products of such aberrations will doubtless escape observation. Other cases, such as some of the dwarf wheats, are almost certainly explicable on this basis. Vilmorin's "ever-splitting dwarf," for instance, may, from genetic comparison with speltoids, be expected to have an odd chromosome, which is probably an excess one.

In view of the slightly irregular chromosome behaviour found in normal type segregates it is obvious that, even apart from the difficulties already pointed out, absolute conformity to expectation based on the formulae presented cannot be hoped for. On the other hand the very good general agreement based on large numbers of observations in these different strains indicates the general validity of the scheme. The problem of the origin of speltoid and other related aberrant forms of wheat and their genetic relationships is so complicated that one can scarcely hope to find a complete solution, and certainly not a simple one. At the same time it is felt that the evidence here presented and the hypothesis developed from it, do explain some of the most striking features.

Summary.

The speltoid strains of wheat found and studied genetically at Svalöf have been grouped by Nilsson-Ehle under three ratio-types, (A), (B) and (C). Heterozygous speltoids of the (A) type produce normal type, heterozygous speltoid, and homozygous speltoid progeny in ratios approaching 1 : 2 : 1, but with considerable deviations from this in some strains. (B) type heterozygotes produce about four or five times as many heterozygous speltoid as normal type progeny, and only a very few homozygous speltoids, which are weak and more or less sterile. (C) type heterozygotes usually produce more normal type than heterozygous speltoid progeny, and again only a few weak and partially sterile homozygous speltoids.

Two (A) type strains of speltoids have been found to have the normal chromosome number, viz. 42, but unusual chromosome arrangements. Heterozygous speltoids of this type are characterised by the presence of a trivalent and a univalent in their pollen mother cells. Homozygous speltoids of this type are characterised by a quadrivalent. The observations on speltoids of this type confirm Winge's (1924) conclusions in the main, and are in contrast to Ekstrand's negative report (Nilsson-Ehle, 1927). Winge's hypothesis is, however, found to be inapplicable without modification to speltoids of types (B) and (C).

The heterozygotes in two strains of (B) type speltoids have been found to possess only 41 chromosomes, which are regularly arranged as 20 bivalents plus one univalent ($20_{II} + 1_I$). These plants form more 20-chromosome than 21-chromosome gametes owing to the halves of the univalent frequently being left out of the daughter nuclei. The progeny ratio characteristic of type (B) is presumably determined primarily by this fact. It is further affected by differential gametic viability and zygotic elimination, both of which are determined principally by chromosome number. The weak homozygous speltoid segregates of this type doubtless have only 40 chromosomes, as have the analogous fatuoid oat segregates (Huskins, 1927), but none were obtained from the 1927 sowings. A homozygous speltoid plant of type (B) which was more vigorous and fertile than is customary in this type was found to have 41 chromosomes, arranged either as $20_{II} + 1_I$ or $19_{II} + 1_{III}$. Two sister progenies of a third (B) type strain were found to be very different from each other. One was similar both cytologically and genetically to the other two strains of this type examined. The other was irregular both

genetically and cytologically. It has evidently changed from the regular (B) type either through chromosome aberration or natural crossing.

In two strains of (C) type speltoids the heterozygotes have been found to possess 43 chromosomes, which are usually arranged as $20_{II} + 1_{III}$. The progeny ratio characteristic of type (C) is presumably determined primarily by the fact that the 43-chromosome heterozygotes form more 21-chromosome than 22-chromosome gametes, and it is modified by differential gametic viability and zygotic elimination similar to that occurring in type (B). A homozygous speltoid of type (C) was found to have 44 chromosomes.

It is concluded that speltoids commonly arise from normal wheat through chromosome aberrations, and that the different ratio-types are determined primarily by differences in chromosome number.

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EVER-SPORTING RACES OF *MYOSOTIS*.

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(With Two Text-figures.)

Two varieties of *Myosotis* with regularly striped flowers have been described by myself in an earlier publication[1]. The facts are briefly that two races, "Star of Zürich" with a white central stripe on blue petals and "Weirleigh Surprise" with a blue central stripe on white petals, have been observed. Only with regard to the former variety is any detailed information available. It has been shown that Star of Zürich does not come true from seed, but behaves genetically as a white, and on the basis of this fact and of the peculiar distribution of anthocyanin obtaining in Star of Zürich it was suggested that this form was a periclinal chimera.

Similar striped varieties have now been observed involving pink and blue; one race having a central pink stripe on a blue petal (this form will be referred to as P.S.B.) and the other a central blue stripe on a pink petal (referred to as B.S.P.). Though there is no reason to suppose that these P.S.B. and B.S.P. plants differ in structure from Star of Zürich, their breeding results are peculiar.

The genetic relationships of the ordinary white-, pink- and blue-flowered plants are as follows. Blue is dominant to white and pink, and in my experience white crossed with pink gives a blue F_1[2], though there may be strains of white which when crossed with pink would give a pink F_1. F_2 numbers are shown in Table I.

It is probable that two factors are concerned:

P, a factor for pink found in all the whites used.

W, a factor which in the presence of **P** gives blue.

The combination **wp** is probably white rather than blue[3], for, though the sum of the F_2 families from white × pink and its reciprocal approximate more closely to a 10 : 3 : 3 ratio than to a 9 : 3 : 4 ratio, this is due to the effect of family 2/27 only. Families 1/27 and 51/26 added together

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[1] *Bibliographia Genetica*, III. 1927, pp. 420–421.

[2] Homozygous strains of pink and white were used.

[3] Although blues and bluish plants have occurred as sports and seedlings from pinks in a particular ever-sporting strain about to be described, all these blues and bluish-flowered plants when bred have thrown pinks.

TABLE I.

Flower colour of parents	F_1				F_2				
	Family	Blue	Pink	White	Parent	Family	Blue	Pink	White
Blues selfed:									
a. (Het. *ex* nat. seed)	—	—	—	11	—	—	—	—	—
b. (From Star of Zürich)	—	34	—	10	—	—	—	—	—
c. (Het. *ex* nat. seed) (From Star of Zürich)	$5/24$	2	—	1	$5^1/24*$	$6/25$	12	—	—
$6^1/25$	$55/26$	5	—	—	—	—	—	—	—
Pinks selfed:									
$6^1/24$	$8/25$	—	42	—	—	—	—	—	—
$6^2/24$	$9/25$	—	53	—	$9^3/25$	$56/26$	—	18	—
Whites selfed or intercrossed:									
Star of Zürich	—	—	—	44	—	—	—	—	—
Star of Zürich × White	$1/24$	—	—	28	$1^1/24$	$1/25$	—	—	—
	$4/24$	—	—	1	$1^2/24$	$2/25$	—	—	44
	—	—	—	—	$1^4/24$	$3/25$	—	—	1
	—	—	—	—	$1^a/24$	$4/25$	—	—	10
	—	—	—	—	$1^b/24$	$5/25$	—	—	2
$5^2/24$	$7/25$	—	—	17	—	—	—	—	—
$2^4/25$	$54/26$	—	—	1	—	—	—	—	—
White × Blue:									
Star of Zürich × Blue	—	4	—	—	—	—	—	—	—
	$3/24$	2	—	1	—	—	—	—	—
$1^1/24 \times 5^1/24$	$10/25$	1	—	3	$10^1/25*$	$52/26$	59	—	18
						Calc.	*57·75*	—	*19·25*
$1^2/24 \times 5^1/24$	$11/25$	1	—	1	—	—	—	—	—
Pink × Blue:									
$6^2 \times 5^1/24$	$13/25$	2	—	—	$13^2/25$	$53/26$	89	31	—
						Calc.	*90*	*30*	—
White × Pink:									
Star of Zürich × Pink	—	12	—	—	—	—	—	—	—
$2^4/25 \times 9^3/25$	$50/26$	2	—	—	$50^1/26$	$1/27$	19	5	10
					Calc. on 9 : 3 : 4		*19·125*	*6·375*	*8·5*
					,, ,, 10 : 3 : 3		*21·25*	*6·375*	*6·375*
	—	—	—	—	$50^2/26$	$2/27$	22	5	2
					Calc. on 9 : 3 : 4		*16·3125*	*5·4375*	*7·25*
					,, ,, 10 : 3 : 3		*18·125*	*5·4375*	*5·4375*
Pink × White:									
$6^1 \times 1^2/24$	$12/25$	3	—	—	$12^2/25$	$51/26$	27	6	10
					Calc. on 9 : 3 : 4		*24·1875*	*8·0625*	*10·75*
					,, ,, 10 : 3 : 3		*26·875*	*8·0625*	*8·0625*
Sum of Families *ex* White × Pink or reciprocal							68	16	22
					Calc. on 9 : 3 : 4		*59·625*	*19·875*	*26·5*
					,, ,, 10 : 3 : 3		*66·25*	*19·875*	*19·875*

* Blue flowered.

or taken separately give a closer approximation to a 9 : 3 : 4 ratio than to a 10 : 3 : 3.

The P.S.B. and B.S.P. plants[1] referred to arose as bud sports in 1924. Five pink-flowered plants and one blue-flowered plant bore sports.
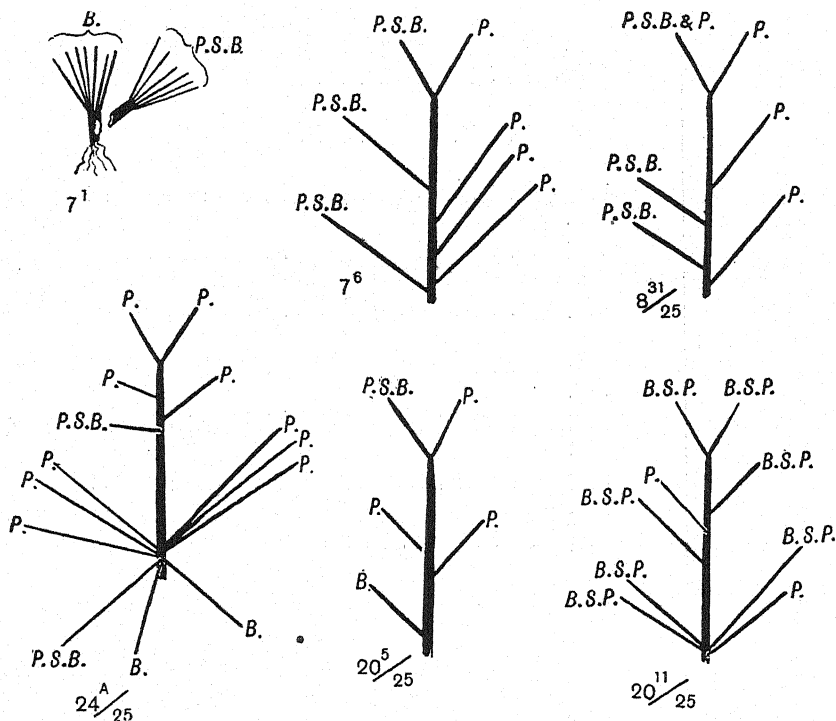
Fig. 1. Diagrams of various chimerical plants showing the types of branches borne. *P.* = a pink inflorescence; *B.* = a blue inflorescence; *P.S.B.* = a P.S.B. inflorescence; *B.S.P.* = a B.S.P. inflorescence.

Two of these pink-flowered plants bore both pink and B.S.P. flowers, one bore pink, B.S.P. and blue flowers and two bore pink and P.S.B. flowers. The blue-flowered plant bore blue and P.S.B. flowers. The various distinct flower types on these plants were selfed. Petals on branches bearing P.S.B. or B.S.P. flowers are frequently not striped and care was taken to label each flower; only the breeding results from regularly striped flowers are included in the tables given.

There are two peculiarities in the results. First, by analogy with Star of Zürich one would expect P.S.B. plants to breed as pinks and B.S.P.

[1] These plants did not occur in experimental material but were observed in various flower borders.

TABLE II.

Family	Plants bearing (branches)											
	Pink	Pink and P.S.B.	Pink P.S.B. and B.S.B.	Blue	Blue and P.S.B.	Blue and B.S.B.	Blue P.S.B. and B.S.B.	Pink and Blue	Pink and Blue and P.S.B.	Pink and Blue and B.S.B.	Pink and Blue P.S.B. and B.S.B.	Pink and Blue B.S.B. P.S.B. White

Original sporting plants:

Family	Pink	Pink and P.S.B.	Pink P.S.B. and B.S.B.	Blue	Blue and P.S.B.	Blue and B.S.B.	Blue P.S.B. and B.S.B.	Pink and Blue	Pink and Blue and P.S.B.	Pink and Blue and B.S.B.	Pink and Blue P.S.B. and B.S.B.	Pink and Blue B.S.B. P.S.B. White
7^1	—	—	—	—	1	—	—	—	—	—	—	—
7^2	—	—	—	—	—	—	—	—	—	—	—	—
7^3	—	—	—	—	—	—	—	—	—	—	—	—
7^4	—	—	—	—	—	—	—	—	—	—	—	—
7^{4a}	—	—	—	—	—	—	—	—	—	—	—	—
7^5	—	—	—	—	—	—	—	—	—	—	—	—
7^6	—	—	—	—	—	—	—	—	—	—	—	—
7^4 and 7^{4a} Pink branches selfed … 29/25	4	1	—	1	—	—	—	—	—	—	—	—
7^4 and 7^{4a} B.S.P. branches selfed … 18/25	14	1	—	32	—	—	—	—	—	—	—	—
7^4 B.S.P. branches selfed … 24/26	3	1	1	3	—	—	—	—	—	—	—	—
7^{4a} Blue branches selfed … 31, 32/25	3	—	—	12	—	—	—	—	—	—	—	—
Star of Zürich ×7^4 and 7^{4a} B.S.P. branches … 22/25	9	—	—	1	—	—	—	—	—	1	—	—
Pink ×7^4 and 7^{4a} B.S.P. branches … 25/25	3	—	—	18	—	—	—	—	—	—	—	—
7^3 B.S.P. branches selfed … 17/25	4	—	—	4	—	—	—	—	—	—	—	—
7^6 Pink branches selfed … 30/25	12	—	—	—	—	—	—	—	—	—	—	—
7^6 P.S.B. branches selfed … 20/25	12	1	—	13	—	—	—	1	—	—	—	—
Star of Zürich ×7^6 P.S.B. branches … 23/25	12	1	—	—	—	—	—	1	—	—	—	1
Pink ×7^6 P.S.B. branches … 26/25	2	—	—	5	—	—	—	—	—	—	—	—
7^1 Blue branches selfed … 27/25	21	1	—	16	—	—	—	1	—	—	1	—
7^1 P.S.B. branches selfed … 16/25	11	1	—	6	—	—	—	1	—	—	1	—
7^1 P.S.B. branches selfed … 17/26	—	1	—	18	—	—	—	—	—	—	—	—
Star of Zürich ×7^1 P.S.B. branches … 21/25	19	1	—	2	—	—	—	—	—	—	—	1
Pink ×7^1 P.S.B. branches … 24/25	3	—	—	—	—	—	—	—	—	—	1	—
7^1 P.S.B. branches × Pink … 49/26	—	—	—	—	—	—	—	—	—	—	—	—

TABLE II (continued).

	No.													
Seedlings:														
20¹/25 B.S.P. branches selfed ...	22/26	—	—	1	—	—	—	3	—	1	—	—	—	3
24⁵/25 Pink branches selfed ...	28/26	30	—	—	—	1	—	—	—	—	—	—	—	—
24⁵/25 B.S.P. branches selfed ...	23, 23·/26	13	—	—	—	2	—	—	—	—	—	—	—	—
20⁵/25 Pink branches selfed ...	25/26	28	—	1	—	1	—	—	—	—	—	—	—	—
20⁵/25 P.S.B. branches selfed ...	18/26	28	—	—	—	—	—	4	—	1	—	—	—	—
20⁵/25 Pink branches selfed ...	26/26	5	—	1	—	—	—	—	—	—	—	—	—	—
20⁶/25 P.S.B. branches selfed ...	19/26	4	—	—	—	—	—	4	—	1	—	—	—	—
24·/25 Pink branches selfed ...	27/26	35	—	—	—	—	—	—	—	1	—	—	—	—
24·/25 Bluish branches selfed ...	30/26	3	—	—	—	—	—	—	—	—	—	—	—	—
24·/25 P.S.B. branches selfed ...	20/25	1	—	—	—	—	—	—	—	—	—	—	—	—
26⁶/25 Pink branches selfed ...	29/26	17	—	—	—	—	—	1	—	1	—	—	—	—
26⁸/25 P.S.B. branches selfed ...	21/26	1	—	—	—	—	—	—	—	—	—	—	—	—
Pink plants selfed:														
16¹/25 ex 7¹ P.S.B. selfed...	31/26	48	—	—	—	—	—	4	—	—	—	—	—	—
18³⁴/25 ex 7⁴ B.S.P. selfed	32/26	55	—	—	—	—	—	—	—	1	—	—	—	—
25¹/25 ex Pink×7⁴ B.S.P.	33/26	27	—	—	—	—	—	8	—	—	—	—	—	—
27³/25 ex 7¹ Blue selfed	34/26	63	—	—	—	—	—	—	—	—	—	—	—	—
29¹/25 ex 7⁴ Pink selfed	35/26	3	—	—	—	—	—	—	—	—	—	—	—	—
30²/25 ex 7⁶ Pink selfed	36/26	63	—	—	—	—	—	—	—	—	—	—	—	—
31¹/25 ex 7⁴ᵃ Blue selfed ...	37/26	21	—	—	—	—	—	—	—	—	—	—	—	—
Bluish plants selfed:														
17¹/25 ex 7³ B.S.P. selfed...	38/26	8	—	—	—	—	—	5	—	—	—	—	—	—
16³/25 ex 7¹ P.S.B. selfed...	39/26	11	—	—	—	—	—	25	—	—	—	—	—	—
18⁴⁵/25 ex 7⁴ B.S.P. selfed	40/26	15	—	—	—	—	—	20	—	—	—	—	—	—
21¹/25 ex Star of Zürich×7¹ P.S.B.	41/26	—	—	—	—	—	—	1	—	15	—	—	—	—
24¹²/25 ex Pink×7¹ P.S.B.	42/26	15	—	—	—	—	—	30	—	—	—	—	—	—
26⁵/25 ex Pink×7⁶ P.S.B.	43/26	12	—	—	—	—	—	27	—	6	—	—	—	—
27¹/25 ex 7¹ Blue selfed ...	44/26	20	—	—	—	—	—	39	—	—	—	—	—	—
31³/25 ex 7⁴ᵃ Blue selfed ...	45/26	7	—	—	—	—	—	35	—	—	—	—	—	—
Blue plants selfed:														
21¹/25 ex Star of Zürich×7¹ P.S.B.	46/26	3	—	—	—	—	—	7	—	—	—	—	—	—
23¹/25 ex Star of Zürich×7⁶ P.S.B.	47/26	4	—	—	—	—	—	19	—	—	—	—	—	—
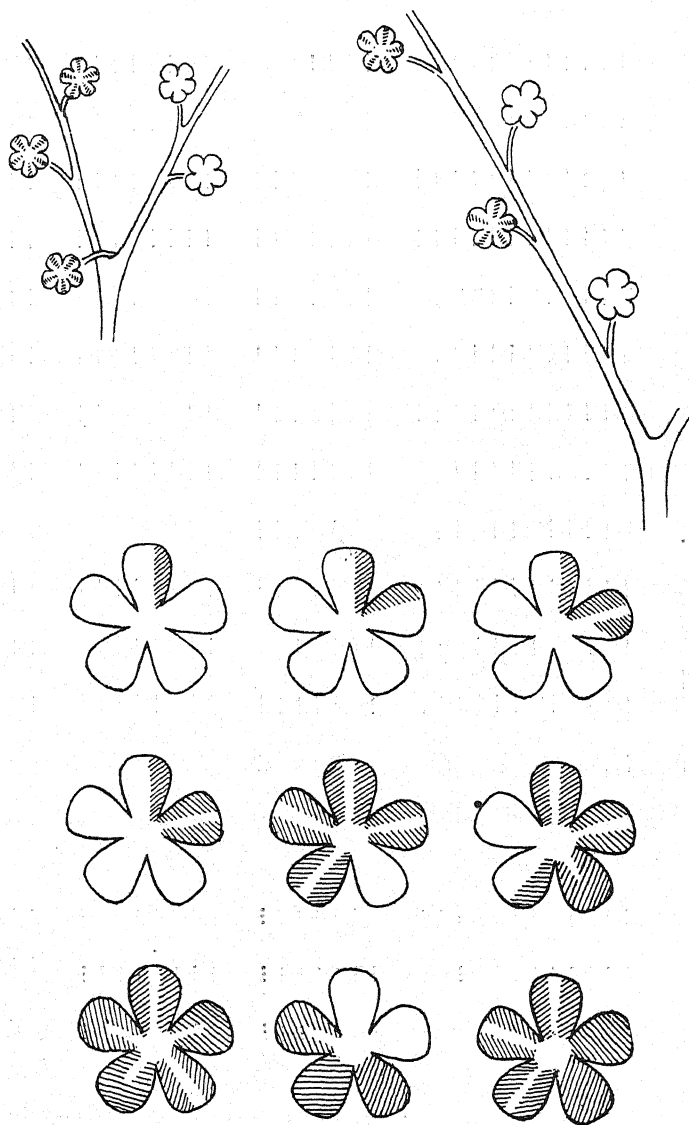
Fig. 2. Showing various sectorial arrangements in inflorescences and in individual flowers on striped-flowered plants. ▨ = blue tissue. ☐ = pink tissue.

plants to breed as blues. As will be seen from the tables this is not the case. Secondly, pink is normally recessive to blue, yet some of the pinks extracted from P.S.B. or B.S.P. can throw blues or bluish[1] plants, and bluish plants can throw pinks.

[1] These plants were paler blue and showed in many flowers persistent traces of pink.

One fact, however, emerges. As in Star of Zürich, striped-flowered plants cannot be bred true from seed; and the striped-flowered branches occur as bud sports on pink- or blue-flowered plants.

The somatic behaviour is strongly suggestive of a chimerical structure. The striped-flowered areas usually occur in regular sectors; occasional petals may remain unstriped; the component tissues of a striped-flowered cutting may separate out as pure pink- and pure blue-flowered branches, and sometimes all four possible stable combinations of pink and blue may occur on the same plant, namely P.S.B., B.S.P., pink- and blue-flowered branches. I believe therefore that the striped-flowered condition is the result of a periclinal arrangement of blue and pink tissues. The irregular breeding results are probably due to the fact that neither the pink nor the blue tissues are pure but that islands of blue exist in the one and islands of pink in the other. The tissues are in fact fine mosaics of blue and pink, the continuous phase being in one case pink and in the other blue. This is a point which is difficult to settle by direct observation since islands of blue may be observed in a normal pink plant and islands of pink in a normal blue. Such fine mosaics have however been observed involving blue and white tissues.

As to the type of mutation which occurred to produce these striped-flowered pink and blue plants, it appears probable to me that it was a plastid, rather than a nuclear, mutation. I have already (Chittenden, 1927) shown some reason for supposing that plastids may be directly concerned with anthocyanin production, and that anthocyanin mosaics may be the result of the existence of dissimilar plastid types in the same plant.

If we assume the various sports which occur in *Myosotis* to be the result of nuclear mutation, we are faced with the old difficulty of a great frequency of mutation in this particular strain of blue and pink plants, from which normal blue- and pink-flowered plants are free. If on the contrary we suppose it to be due to plastid mutation, then, if the inheritance of plastids be biparental in this case, it is only necessary to assume one mutation, all the sports observed being due to the subsequent somatic segregation of the two dissimilar plastid types. There is then no longer any difficulty in interpreting cases where a blue sport from a pink plant subsequently throws pinks, and where pinks are throwing blue seedlings.

It is probable that after a period of vegetative propagation as prolonged as that to which Star of Zürich has been subjected these plants will also become stable somatically and genetically.

THE AGOUTI COLORATION OF THE MOUSE (*MUS MUSCULUS*) AND THE RAT (*MUS NORVEGICUS*).

By F. W. DRY, D.Sc. (*Leeds*).

(With One Text-figure.)

In a recent paper[1] on the coat of the Mouse mention was made of preliminary observations on the agouti colour pattern. It is now desired to record certain details of pigmentation in the wild type of the Mouse and the Rat which lend support to the conclusions of Onslow and Wright as to wherein lies the difference between agouti and non-agouti. The features of hair growth and form dealt with in the other paper need not now be recapitulated, and few new terms will be employed. Attention may be directed especially to the section on hair-type-associations within single follicles, and also to the plates. References are cited in the previous paper.

The skins used were from captured animals with the exception of the mouse skins in which the third or later pelages were examined. Those were from black agouti stock obtained from a fancier, and the same applies to the living rats used for observations on hair succession. The work has been confined almost entirely to mice that had not grown more than the first three hair generations, and to rats bearing only the first and second pelages. The points brought out depend upon the existence of differences rather than the extent of those differences, and it is not deemed necessary to present tables of measurements. Where the conformation of the body is the same no difference between the sexes has been detected in any hair character. The description of pigmentation in the Mouse would be largely applicable to the Rat, but the details have been studied more thoroughly in the smaller animal. In the Rat measurements of hairs have been made only for the region of the mid-dorsum.

THE MOUSE.

A mouse of the wild colour type differs from a black one in that upon the colouring of the black one is superimposed a sub-apical or apical light banding in many hairs. On the mid-dorsum of both types the largest overhairs (Type A and the bigger Type A–B)[2] are on the

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[1] *Journ. Gen.* XVI. 287.

[2] For the meaning of these terms see *Journ. Gen.* XVI. 1926, pp. 287–340.

average less darkly pigmented in the tip and the extreme end of the shaft, in both cortex and medulla, than the smaller overhairs. On the lower parts of the body of blacks the amount of pigment in the distal parts of the hairs is strikingly less in the larger overhairs, this feature becoming more marked more ventrally, but in light animals all the hairs of this region may be lighter at the apical end than more basally. The lightness under consideration is due to the small amount of black pigment; there is no yellow pigment.

In wild mice there is some black pigment apical to yellow on the dorsum, but in lighter parts of the coat this may not be so. Once yellow has given place completely to black at the proximal end of the band no further yellow has been detected in the remainder of the hair. In banded hairs of the same pelage relations can be defined between the length of the band and the type and size of the hair. On the dorsum, broadly speaking, the smaller the overhair, the longer the band. At positions successively more ventral from the middle of the side the bands in corresponding hairs become longer, starting more distally and ending more proximally, and many hairs are banded which more dorsally would be black. In wild animals with very light bellies the agouti band may contain little or no yellow, and in such animals the light apical regions in which the lightness is that of Self, as distinct from that of the agouti banding, are long. Studies have been made in considerable detail of pigmentation at different points between mid-dorsum and mid-venter in such a light animal. By taking into consideration the two sorts of lightness it is possible to explain both the transition in colour details shown by corresponding hairs in different regions, and the transition revealed in a series of hairs, large to small, of the same type in the same region. On the abdomen of this mouse there was a long white apical region in the biggest overhairs, both an apical and a sub-apical white region in hairs of intermediate size, and a long white apical region again in the smallest overhairs. In these the intervening black region of the second group is no longer present. The two white regions, we may so regard it, have merged into one. The agouti band extends a greater absolute distance from the tips of awls on the venter than in those of the dorsum, and as the hairs of the venter are a good deal the shorter it comes about that on the abdomen the band may occupy a very large part of the hair.

Black pigment, in both cortex and medulla, is always granular. In heavily pigmented parts of the coat yellow pigment exists as granules in the distal part of the septum or septule, and in diffuse form in a

definite region embedded in the proximal part. This portion is more or less globular in shape and is usually flattened; the terms "globule" and "globular pigment" will be employed (Fig. 1). These globules cannot depend for their existence upon any process of actual pigment formation. They can be recognised in the unpigmented parts of hairs in the wild type and throughout the medulla of albinos, except it may be where this is very narrow at the ends or at a constriction. When pigment is absent it may be presumed that the globules are rendered visible by
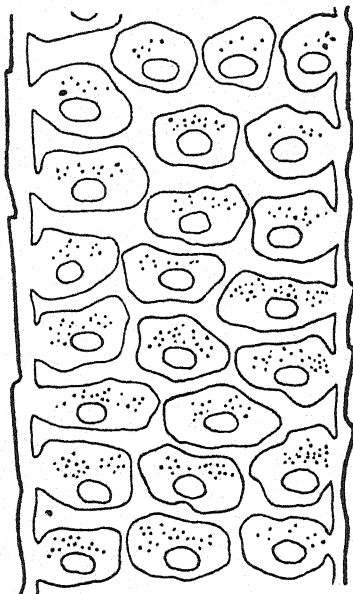
Fig. 1. Banded auchene, basal end of band with a light yellow globule in each septule in company with black granular pigment. Camera lucida drawing of optical section. (× 1200.)

difference in refractive index. Staining with picric acid makes them rather more easy to see. They are found, equally widespread over the length of the shaft, in light colour types, for example Champagne, in association with dark granular pigment. Where there is full black pigmentation I fail to detect these globules either in hairs examined whole or in sections. In agouti bands that are pale black, with no yellow, there may or may not be globules. Yellow of both kinds and black may all exist together in the same medullary unit. In the very bright yellow bands of some animals diffuse yellow, while more concentrated in the globule, appears also to pervade both medulla and cortex. For some distance at the proximal end of a band the amount of granular yellow and the depth

of the yellow shade of the globules are both reduced. Yellow mice of the kind called Red by the Fancy have both kinds of yellow pigment.

As Bateson and Miss Durham found, black, chocolate, and yellow pigments, in the order named, are successively more soluble in caustic potash. On the whole granular yellow dissolves more easily than the globular kind. Bateson and Miss Durham believed the hairs of wild mice to contain pigment of all three colours, but I cannot convince myself of the presence of chocolate, and think they may have been referring to what I call granular yellow. This pigment, in both agouti and red animals, can be distinguished under the microscope from the pigment of chocolate mice.

Colour in Relation to Structure.

The comparisons to be made are between hairs of the same pelage. In considering the relation between the size and colour of a hair importance is attached not to absolute size, but to comparative size for the particular pelage as an expression of relative rate of output—average rate for the whole hair or rate for a particular part—of hair substance from the follicle. Many banded overhairs of a later pelage are bigger than black ones of the first.

Observations on hair development and arrangement have not indicated any relation, except in so far as position and size can be correlated, between the position of a hair in a small region of the same shade throughout and the colour of the hair. In the baby coat, it may be recalled, the bigger the hair type, the earlier does development start, and I am satisfied that this applies also to differences of size between zigzags. Adjacent follicles both growing zigzags may at the same moment be producing black pigment in the one case and yellow in the other, and this is true for the second pelage when all the hairs in the same area are starting to grow simultaneously. Hairs grow, of course, only at the basal end. There is no indication that in the matter of pigment formation one follicle has any influence upon another.

I have no exact data on the rates of growth of different parts of the same hair, but observations on partly developed hairs in a series of skins from the same litter point to growth rate (and probably rate of output per unit of follicular tissue producing hair substance) being higher in the more distal parts of overhairs, where a large cross-sectional area is quickly attained, than nearer the basal end, and to a high rate also for Segment I of zigzags. As for the constrictions, it is easy to suppose that they mark a reduction of output, but an examination of the stages of development reached by a large number of zigzags from the same

part of the back of a few animals about 6 days old leads me to think that the constricted region is formed quickly, and that the rate of output of hair substance at that time is not so low as might be supposed. In view of observations reported in my earlier paper (pp. 305–6), and of the simple fact that the region of greatest cross-sectional area is not very far from the tip, it is suggested that the growth rate attains its maximum quickly and afterwards gradually falls.

The hair types are considered from the mid-dorsum first. Monotrichs (Type A) and Type A–B are always "black," that is, possess no agouti band, either pale black or yellow. Between black and banded awls (Type B) there is but little difference in total length, though the former tend to be longer. Black awls are on the average of distinctly greater maximum breadth and have more inside septules. It may further be stated that in banded awls the figures for these two characters average more in those containing some black pigment throughout the light area than in those with the pigment exclusively yellow in the middle region of the band. In hairs with a pale black band this has a similar position to the part of a yellow band where the yellow shade is the deepest.

Between black and banded auchenes (Type C) there is no appreciable difference in total length, but the blades of black hairs, as judged by length, maximum breadth, and number of inside septules, are on the average the bigger. In the lighter animals black auchenes are rare, and the great majority of hairs of this type have yellow bands starting close to the tip and with a long region free from black pigment. In some of the bigger auchenes the band is short, and there is a longer black region distal to the band similar to that found in awls. In very small auchenes the band is short, beginning early but soon coming to an end. On the mid-dorsum it is the rule for full black pigmentation to be restored before the constriction of the auchene is reached.

In zigzags (Type D) total length has been found a sufficient measure of size. The band is confined to the distal segment. The length of the band increases with the total length of the hair. The increase is a gradual one, just about keeping pace with total length, save that in the first pelage of the rather light animals specially studied the length of the band at first increases rapidly with additional units of total length, being zero in the few shortest hairs of all. In the later pelages hairs with very short bands are rarer. In light animals it is only short bands that have black pigment throughout, while at the other extreme in some animals the largest zigzags are characterised by very deep orange bands.

The length of Segment I, although it may be very short in the shortest hairs of all, is at or near its maximum in quite short hairs, after which, for successively greater lengths, the length of this segment gradually becomes less. A gradual falling-off may be sustained up to the greatest lengths. It is true that the shorter hairs are apt to have one segment fewer than the longer, but even so the falling-off in length of the segment is found in a series of hairs grouped according to total length in all of which every hair has the same number of segments.

On the side and venter monotrichs never show an agouti band, though there may, of course, be an apical light region characteristic of self colour. The band may, however, be found in Type A–B which on the back is not banded.

In auchenes from the side and abdomen of the light animal, to which particular attention was paid, the pale black end of the band was found nearly always to reach the constriction, and the length of the band, which extends over the whole of the blade, increases or decreases with the size of the blade. Here is a direct—not an inverse—relation between the size of the band and the size of an overhair, a state of affairs resembling that found in zigzags on both the dorsum and the side.

In zigzags on the abdomen of the same animal there is no dark pigment at the apical end of the hair, and the basal end of the band coincides closely with the proximal end of Segment I. As this segment tends to be long or short with, respectively, small or great total length of the hair, we find the longer bands in the short zigzags. This, curiously enough, is the opposite of what occurs on the mid-dorsum and mid-side where there is some black pigment at the proximal end of the distal segment. In the cases just discussed the coming to an end of the band is thus associated with the constriction.

The Colour and Size of Hairs of the same Follicle.

As recorded in the earlier paper, definite relations exist in regard to hair type in the series of products of the same follicle. In alphabetical order from A to D the hair types are spoken of as successively lower, or from D to A as higher. Where a hair is of a higher or a lower type than that of the preceding generation the terms "step-up" and "step-down" respectively are employed, and "level" when there is no change of type. In G_1–G_2 pairs step-ups are common, step-downs very rare. In pairs belonging to two consecutive pelages later than this step-ups are less numerous and step-downs more in evidence.

When the presence or absence of the light band in hair partners from agouti animals is considered it is found possible to generalise about

the relations between companion hairs just in the same way as in respect of type. Save in Type D a hair black throughout is looked upon as higher than one that is banded. The same hair is often a type level but a colour step-up. When similarities or differences displayed by members of the same hair bundle are examined orderly relations can be established not only in regard to broader features, but in considerable measure in respect of details of both structure and pigmentation.

Most data were secured from the mid-dorsum of animals bearing the first two pelages. The results derived from 6 mice, from equal numbers of pairs of hairs, of which at least one was an overhair, are given in the following summary.

G_1 and G_2 both overhairs
   Both hairs black
      Both Type A (G_1 lost) ... 43
      Neither Type A 125
                          168
   G_1 banded, G_2 black 89
   Both hairs banded 158
                                   415
G_1 Type D, G_2 overhair, both banded   185
                                   600

Occasional type step-downs occur, but I have not found a G_1–G_2 colour step-down. As stepping-up in colour from banded to black is common, and as practically all zigzags are banded (save in very dark animals), the result is that the number of black hairs is greater in G_2 than in G_1. As, however, many zigzags are partnered by G_2 auchenes with large bands it is easy to understand how the proportionate area of yellow to black may remain much the same.

As examples of the generalisations that can be made about the members of type-associations the following particulars are given for pairs composed of two auchenes, a zigzag and an auchene, and two zigzags. When the partnerships G_1 C–G_2 C and G_1 D–G_2 C are compared, the second pelage auchenes are seen to be appreciably larger on the average in the first case. The average length of the band is a trifle less; the longer bands have much the same length in both groups, but the larger set of hairs includes some with short bands. In the type-association G_1 D–G_2 C it is possible to say that the bigger the first pelage hair, the bigger on the whole is that of the second pelage. In this hair-partnership the zigzags are amongst the biggest of their type in the first pelage, some of them being larger than any of the same type that have zigzags for companions.

In the partnership G_1 D–G_2 D there is a high degree of correlation between the total length of the two hairs, and as is implied in what

has already been said, between the length of the agouti bands. The second pelage hair is always the longer, but the difference in length between G_1 and G_2 becomes on the whole less with increasing length of G_1. With this one associates the fact that the G_1 zigzags begin their development by no means at the same time, although they finish growing within about one day of one another. The longer hairs have several days more in which to grow than the shorter, but in G_2 all start their growth and afterwards bring it to a close very much together.

About 1 per cent. of the pairs of zigzags from the mid-dorsum in the animals under consideration contain a G_1 hair black throughout or with a pale black band. All these hairs are very strikingly short. Their G_2 companions all possess yellow bands.

Comparisons have been made for the several regions studied between mid-dorsum and mid-venter of the points on the shaft of overhairs where the band begins and where it ends in hairs grouped according to type-association. When first pelage hairs are being compared the groups distinguished are G_1 B (banded), G_2 B (black); G_1 B–G_2 B (both banded); G_1 C (banded), G_2 B (black); G_1 C–G_2 B (both banded); and G_1 C–G_2 C (both banded). When the comparison is between second pelage hairs the type-associations, in which all hairs are banded, are G_1 B–G_2 B, G_1 C–G_2 B, G_1 C–G_2 C, and G_1 D–G_2 C. Where differences can be recognised in the point at which the band begins this point tends to be nearer the apical end in the successively named sets of hairs. That is to say, with decreasing average size of the hairs of the group the band begins nearer the tip. The proximal end of the band tends to be less far from the tip in the smaller hairs. Where a set of awls has shorter bands than a set of auchenes this is due to the later beginning of the band in the awls which more than counterbalances any difference in the position at which the band ends. It has already been stated that in zigzags, as in most auchenes, the band begins very near the apical end of the shaft.

Comparisons of the position of the band in the hair were also made for the several areas examined between hairs of the same type of the first pelage and of the second. In the second pelage the hairs are of course larger. Except in so far as this may be prevented in awls by a longer apical black region, the band, extending as it does a greater distance proximally, is longer in G_2.

In an animal bearing the third pelage that has been specially studied type step-ups from G_2 to G_3 are very scarce, colour step-ups rather more plentiful; type step-downs are common, and colour step-downs do just occur. Very occasionally indeed a G_3 hair is a step-down on both counts.

The relations of companion hairs are just what one would expect after becoming familiar with the orderly relations existing in pairs of hairs of the two earlier pelages. A single example will suffice. G_1 zigzags in the associations G_1 D, G_2 C, G_3 D and G_1 D, G_2 C, G_3 C are all large, but they are longer in the latter. The G_3 step-down zigzags, those in the first of the two associations just named, are amongst the biggest G_3 zigzags, so that both zigzags in these bundles are amongst the biggest of their pelage. The rarity of the intermediate type, Type C–D, has been emphasised in the earlier paper. The sharp break in structure between Type C and Type D is especially brought home to one in contemplating zigzags of G_1 and G_3 in partnership with a G_2 auchene.

The Rat.

The Hair Types.

The hairs are built on the same plan as those of the Mouse. The medullary units are of much the same size. Very broad overhairs, broader than any occurring in the Mouse, show a larger number of septules in a traverse from side to side than is ever found in hairs of the smaller animal. The largest overhairs in adult rats approximate in structure to the vibrissae of the Mouse in that the cortex is very thick, and that the septules are more meagre objects than in smaller hairs.

As in the Mouse the hairs of the dorsum are classified according as they have no constriction, one constriction, or several. The largest hairs of all, Type A, more or less circular in cross-section, and with very long tips, are again called monotrichs, for I am satisfied that, as happens in the Mouse, when one of these hairs of a pelage later than the first completes its growth, its predecessor in the follicle, also of this type, is caused to fall out. These hairs are doubtless sensory in function. Between them and awls (Type B) it has not been necessary for the present purpose to fix a dividing line because on the dorsum of the agouti Rat all overhairs above Type C have been found to be black throughout. It may be mentioned that in full-grown rats the very broad awls for the greater part of their length have but little pigment in the septules of the median region. Though the hair types are different, one is reminded of the tendency to weak pigmentation in the apical parts of Types A and A–B in the Mouse. Often in the constriction of overhairs, where this occurs, there are two rows of septules throughout, and the narrow region may even include inside septules, but these hairs are all classed simply as auchenes.

The smaller overhairs, awls and auchenes, do not have the pronounced concavo-convex cross-section that they have in the Mouse. The cross-section is oval or slightly concave on one side. At the most a U-pattern in the margins of the cuticular scales has been found to be only slightly developed. Very commonly the scale arrangement, similar on both surfaces of the hair, is on an imbricate plan of a kind much more common in mammalian hair than the deep U-pattern that is the rule on a large part of the ectal surface of awls and auchenes in the Mouse.

The smaller zigzags of the first pelage are of much the same size as corresponding ones in the Mouse, but the larger zigzags are appreciably the bigger in the Rat, and zigzags grown by the adult animal are distinctly longer than corresponding ones in the Mouse. The largest zigzags will often have just a few septules as is the case in the Mouse. Occasional hairs are truly intermediate between Types C and D, but it is to be emphasised that these are but few in number in both the pelages particularly studied. The rarity of Type C–D has often been dwelt upon as a striking feature in the Mouse.

There is a further resemblance to the Mouse in the existence of very short hairlets, nothing intermediate between them and a short typical zigzag having been found on the dorsum. Again, they are found more especially close to monotrichs. A hairlet and a zigzag have been found as companion hairs in the same follicle, but even when two pelages have been grown the vast majority of these tiny hairs are alone in their follicles, and it is probable, just as is true in the Mouse, that these follicles do not necessarily give rise to a hair when the rest of the follicles participate in the growth of a hair generation.

If we compare the whole series of hairs of the same pelage from the mid-dorsum of the Mouse and the Rat, in the larger animal the greater range in hair size between the smallest and the biggest individual fibres is very noticeable. With this fact it will be possible to correlate the differences between the two rodents in the details of the agouti coloration.

Hair Development and Succession.

The developmental phases of individual hairs are the same in the Rat as in the Mouse. I have not found residual pigment in the basal saccules, but in one wild mouse I also failed to find it. The G_1 hairs grow singly, and, again as in the Mouse, with the growth of G_2 one new hair, and one only, is produced in every follicle. In the animals studied which bore the second pelage it was found that the G_1 hairs were not retained in such numbers as is the rule with the Mouse.

Observations on a few living rats kept in confinement have revealed the same kind of orderly progress in the growth of a pelage as that which has been illustrated for the Mouse by a series of charts[1]. For both the second and the third pelages the process was found, however, to be less hurried. In both of them growth starts on the venter and gradually spreads to the dorsum, but the area on which hair is growing at any particular time is more limited than is usual in the Mouse. Thus on the side growth may be restricted to a strip about half an inch wide running along the length of the body. The dorsal margin of this strip advanced only slowly, and in both G_2 and G_3 its position was defined by a sharp difference in length between the hair of the newer and of the older pelage. On the head, as in the Mouse, there was apt to be delay in the growth of a pelage later than the first.

In one respect the second pelage of the Rat stands in sharp contrast to that of the Mouse. In G_2 in the Rat a number of new follicles are founded. These, I believe, always contain zigzags, and the vast majority of these zigzags, possibly all of them, are short and have no band. The animals with which I worked were not specially dark, but of about average shade, and nearly all G_2 zigzags growing as companions to G_1 hairs possessed a pale black or yellow band, though occasional pairs of black zigzags were encountered. The G_2 "additional" zigzags I estimate at about one-third of the underfur hairs of their pelage. A further point is to be noted about their development. Once the growth of the pelage is well under way the hairs growing in the old follicles all attain very similar degrees of development about the same time, but those growing in new ones tend to lag behind, even far behind, pointing to appreciable differences in the times of initiation of their development.

Many of the additional zigzags are among the last G_2 hairs to complete their development. Of the other G_2 hairs the larger ones finish growing somewhat later than the smaller ones, the monotrichs being especially late in becoming club-hairs. At the beginning of the growth of G_2 the bigger hairs likewise have an advantage over their smaller neighbours in a slightly earlier start. Not much, however, of the greater size of the hairs in question is to be attributed to the longer growing time. In the second pelage of the Mouse these differences in commencing and completing growth are not found. In the features of hair development just discussed the Rat may be regarded as less specialised, and the same applies to what has been recorded about the shape of the cross-section and the scale pattern of small overhairs.

[1] Cf. *Journ. Gen.* XVI. 1926, pp. 338–340.

Type, Size and Colour in Hairs of the same Pelage.

It has already been stated that the hairs of the same pelage cover a greater range of size than in the Mouse, and the part of this range in which yellow pigment makes its appearance is more restricted. In rats by no means dark I have never found Type B to be banded, and black auchenes are more plentiful than in mice of similar shade. They are the larger hairs of their type, and on the whole in the animals studied are outnumbered by the banded ones. In G_2, where many auchenes are succeeding zigzags, banded hairs are clearly in the majority in Type C. Black zigzags are far more numerous than in any but dark mice. In G_1 about half the zigzags, the smaller ones, are black. In G_2, as has already been recorded, very few of the zigzags that partner the earlier zigzags lack the band, but the black additional zigzags, forming about one-third the total of the G_2 underfur hairs, make up the number of black zigzags to much the same as it was in the baby coat.

In both pelages studied zigzags of the group with no band, with pale black band, and with yellow band are successively of larger average size. In the banded hairs on the whole the longer the hair the longer the band. With increasing total length the number of constrictions tends to increase, and there is a steady falling-off in the length of the distal segment, save that in the biggest G_2 zigzags this segment tends to be somewhat longer, leading up to the condition found in the intermediate type, Type C–D, in which the distal segment approaches in size the blade of a small auchene. All this is very similar to what is found in the Mouse. In the G_2 additional zigzags, the shortest of their pelage, there are often only two segments and the basal curl—in contrast to five in the big zigzags—and Segment I is very strikingly long. This matter of the length of segments in zigzags is one which might be commended to the attention of a mathematician.

Hair-type-associations within single follicles.

Here again the story runs parallel to that for the Mouse. There is the same phenomenon of stepping-up, but I have not found stepping-up in both colour and type in any pair of hairs, nor such a big jump as in G_1 D–G_2 B which is met with now and again in the Mouse, and in pairs of overhairs stepping-up of either kind is less frequent in the Rat. It is helpful to link up these facts with the greater range of size in the hairs of the same pelage of the Rat.

It is in pairs of zigzags, with the wide range of size that these hairs

have in the Rat, that differences in colour-associations can be studied best. As in the Mouse, a black zigzag is regarded as lower than a banded one. The following partnerships occur, G_1 being named first: yellow and yellow, pale black and yellow, black and pale black, black and black. In these successive groups the average size of the hairs gradually decreases for each pelage, and in the first three groups the length of the G_2 yellow band gets steadily less. The colour-associations named are the only ones recorded for pairs of zigzags, and I have not yet found G_1–G_2 step-downs of any sort in the Rat.

In the example just given of differences in hair size in pairs of zigzags grouped according to their colour-associations it is possible to supplement what has been recorded for the Mouse in this matter. In the other associations, namely, D and C both banded, 2 C's both banded, C banded and C black, 2 C's both black, 2 B's both black, the facts about size are equally orderly, and merely a repetition of those for the Mouse.

DISCUSSION.

Two main points stand out. One is the correlation between the presence and the size of the agouti band with hair type and size; the other, the orderliness of the relations involved in the type, size, and colour of the successive products of the same follicle.

The details of pigmentation may be interpreted in accordance with the views of Onslow and Wright upon the manner in which the agouti ticking is produced. Following Wright we may postulate a black-producing enzyme **B**, and an inhibitor of black, **I**, to which is also attributed the power, directly or indirectly, of producing yellow. Wright's suggestion is that yellow is formed by a third substance, itself produced by the action of **I** upon **B**. The threshold of pigment production for **B** is supposed to be lower than for **I**. In hairs on the dorsum with pale black bands **I** would seem able partly to inhibit the formation of black without producing any yellow pigment, and on the venter of light animals **I** can on this view sometimes inhibit black completely in a large part of a hair while unable to give rise to any yellow at all. The presence of agouti bands in the lighter parts of the coat in hairs which on the dorsum would be black, and their greater length in the lighter regions in comparable hairs, may be put down to a lower chromogen concentration.

The facts recorded about the agouti coloration are so orderly that their significance must surely be simple, but to suggest a particular interpretation is somewhat hazardous.

It seems likely that any part of any hair is potentially black or yellow, but that different degrees of activity of the hair-producing tissue give different results in regard to colour. With successively increasing degrees of activity I believe the colour produced is (1) "low-rate" black, (2) yellow, (3) "high-rate" black. I would classify the black pigment of different regions of the hairs and of different hair types as follows.

Black proximal to yellow is low-rate. The first black pigment formed in any hair, very soon after growth starts, is low-rate. The small amount of black distal to yellow in zigzags and most auchenes on the dorsum is probably all low-rate.

In overhairs where the distal black region is longer the black pigment adjacent to the band distally is high-rate. On this view, somewhere between the extreme apical black and the black next to the band there must be a transition from low-rate black to high-rate black. It is suggested that this transition is passed through very quickly so that no yellow pigment is formed, nor is there any observable diminution in the quantity of black pigment at this point.

In the large overhairs that are black throughout, the region of high-rate black is long, but the pigment in a long proximal region, which region in awls may be very similar in breadth in both black and banded hairs, is regarded as low-rate black. On the view adopted there must be a transition from high-rate to low-rate, but in the biggest hairs there is no sign of yellow pigment or of a pale black region, though the existence in some hairs of a short pale black region is a pertinent fact. It is suggested that the enzyme **B** is more stable than the enzyme **I**, and that this greater stability of **B**, which is thought of as having thoroughly got the upper hand in the high-rate region, enables the transition to low-rate black to be passed with sustained full black pigmentation.

In the very small entirely black zigzags we have low-rate black throughout. In zigzags with a pale black band, this band, as I view it, is the product of the greatest activity attained by the particular follicle.

With regard to the hair-type-associations it may simply be said that stepping-up in colour, which is so frequent in G_1–G_2 pairs, is an expression of the difference of activity of the follicle in the two pelages.

This discussion may suffice, being speculative already, but the explanation suggested seems to me consistent with the numerous details that have been presented. The conclusion to be emphasised is this, that black pigment is formed under two different sets of conditions, and that yellow pigment is produced in intermediate conditions.

SEXUAL DIFFERENCE OF LINKAGE
IN *GAMMARUS CHEVREUXI*.

By J. S. HUXLEY.

(With One Text-figure.)

(1) *Introduction.* In previous papers it has been shown that the genes at the **b** and **c** loci (red and albino eye) of *Gammarus chevreuxi* are linked. The previous data were all from F_2 material; the present data are from back-cross tests (**BC.bc** × **bbcc**, and **Bc.bC** × **bbcc**, and reciprocals). These permit of analysis of crossing-over in the two sexes separately.

The essential results are presented in Table I. The following points may be specially noted.

(2) *Sexual difference in linkage intensity.* The linkage is much more intense in the male than in the female, the c.o.v. for males being 25·4 per cent., that for females 50·6 per cent.

It has been suggested by Seiler (1922), Kuwada (1919) and Goldschmidt (1923) that a form of linkage would be obtained if whole chromosomes tended to remain through the reduction divisions associated with each other in the same way in which they entered the zygote. If this were the case, then the 50 per cent. c.o.v. of the female would indicate that no such association occurred in the female sex. A cytological sexual difference in chromosome association has been noted in *Lymantria* by Seiler and Haniel (1922).

Until other linked genes are discovered in *Gammarus* it will not be possible to say whether this hypothesis is the true one, or whether linkage is of the normal type, and simply weak in the female. If the c.o.v. were always 50 per cent. in the female whatever its value in the male, the hypothesis of association of whole chromosomes would be supported. On the whole it is best to assume weak linkage of normal type in the female unless other data make it necessary to reconsider the question.

It is worth recalling that the sexual difference in linkage intensity is very similar to that in *Paratettix* between the factor Θ and the large

TABLE I. *Summary of linkage results between* **B** *and* **C**, *for male and female respectively.*

	No. of broods	b	r	a	bn	rn	c	T.	N.C.O.	C.O.	N.C.O. +C.O.	C.O.V. %
A. ♂ parent heterozygous												
I A. Coupling **bc.bc** ♀ × **BC.bc** ♂												
I A 1. No **www** offspring	91	668	245	912	411	161	—	1825	668	245	913	26·8
I A 2. **W**+**www** expected 1 : 1	124	417	162	543	166	32	563	2257	828	323	1151	28·1
I A 3. **W**+**www** expected 3 : 1	106	523	130	586	314	96	213	1650	689	162	851	19·0
I A 4. All offspring **www**	46	—	—	—	—	—	395	805	314	96	410	23·4
Total ♂ coupling	367								2499	826	3325	24·9
II A. Repulsion **bc.bc** ♀ × **Bc.bC** ♂												
II A 1. No **www** offspring	13	47	71	130	44	150	—	248	71	47	118	39·8
II A 2. **W**+**www** expected 1 : 1	37	42	139	205	11	25	168	748	289	86	375	22·9
II A 3. **W**+**www** expected 3 : 1	16	32	112	129	51	105	36	345	137	43	180	23·8
II A 4. All offspring **ww**	17	—	—	—	—	—	137	293	105	51	156	32·7
Total ♂ repulsion	83								602	227	829	27·1
Total for ♂	450								3101	1053	4154	25·4
B. ♀ parent heterozygous												
I B. Coupling **BC.bc** ♀ × **bc.bc** ♂												
I B 1. No **www** offspring	28	149	136	253	165	197	—	538	149	136	285	47·7
I B 2. **W**+**www** expected 1 : 1	85	197	221	418	32	19	413	1611	362	418	780	53·6
I B 3. **W**+**www** expected 3 : 1	29	93	87	164	159	160	66	461	125	106	231	45·9
I B 4. All offspring **ww**	36	—	—	—	—	—	282	601	159	160	319	50·2
Total ♀ coupling	178								795	820	1615	50·8
II B. Repulsion **Bc.bC** ♀ × **bc.bc** ♂												
II B 1. No **www** offspring	6	45	46	90	53	63	—	181	46	45	91	49·6
II B 2. **W**+**www** expected 1 : 1	5	55	63	104	18	19	100	438	126	108	234	46·4
II B 3. **W**+**www** expected 3 : 1	14	65	59	107	18	14	40	308	78	83	161	51·5
II B 4. All offspring **ww**	5	—	—	—	—	—	35	67	14	18	32	56·3
Total ♀ repulsion	30								264	254	518	49·0
Total for ♀	208								1059	1074	2133	50·4

multiple allelomorph system *A, B, C*, etc. (Nabours, whose data were analysed by Haldane (1920)).

This is of interest since Haldane has advanced the hypothesis that in this animal the linkage mentioned may be between whole chromosomes. On the other hand, neither Palmer (1926) nor Dence (unpublished data) could find any evidence of association between whole chromosomes in *G. chevreuxi.*

(3) *Possible effect of reduced recessive viability on observed cross-over values.* As long ago pointed out by Morgan and his pupils, any differential viability between recessive and dominant in linkage tests will cause the observed ratios to deviate from the true genetic ratios. In back-crosses involving coupling the observed cross-over value will be lower than it should be, in those involving repulsion it will be higher. If the viability of **bbC** were 5 per cent. lower than that of **BbC** animals in *Gammarus*, and the true genetic cross-over value were 25 per cent., the observed values would be very close to 24 and 26 per cent. for coupling and repulsion tests respectively.

As a matter of fact, the figures given by Allen and Sexton (1917) do not indicate any marked differential viability of red as against the normal black. Excluding the three cases of probable linkage mentioned by me in an earlier paper (Huxley, 1921, pp. 232–3) their totals when added up are found to be as follows: (*a*) Expected ratio 3 : 1:—(i) wild type : red, 2074 : 699; (ii) black, no white : red, no white, 174 : 87. Total *B* : *bb*, 2248 : 786, = 2·86 : 1. (*b*) Expected ratios 1 : 1:—(i) wild type : red, 1020 : 1013; (ii) black, no white : red, no white, 107 : 98. Total *B* : *bb*, 1127 : 1111, = 1·014 : 1. All in all, therefore, the reds emerge as slightly *more* viable than the blacks, and curiously enough this is more marked with wild type black than with no-white black.

The actual figures obtained by me for c.o.v. in the male are, for coupling crosses 25·3 per cent., for repulsion crosses 27·4 per cent. This is consonant with the idea of less viability of the recessives; but the difference is slight, and in view of the above evidence of Allen and Sexton is presumably not significant.

It will be seen that there is a slight deficiency of **cc** individuals throughout, both as regards with-whites and no-whites, and both in males and females. To this rule there are but two exceptions, both of them in groups with comparatively small numbers. There is a similar slight deficiency of **ww** individuals. It is presumably an accident that both the exceptions to the rule concerning **cc** deficiency occur in the female series. If this is so, the general conclusion is that both **c** and **w**

when homozygous are slightly less viable than wild type, to the extent of about 3 per cent.; and that the excess of **ww** individuals noted in the F_2 linkage experiments (Huxley, 1921, p. 231) was not significant.

The figures for the segregation of the other factors are given in Table II.

TABLE II.

I	II	III	IV	V	VI	VII	VIII	IX
						$bn+$	$b+r+$	
	$b+r$	a	$bn+rn$	c	$b+r+a$	$rn+c$	$bn+rn$	$a+c$
I A 1	913	912	—	—	—	—	913	912
I A 2	579	543	572	563	1122	1135	1151	1106
I A 3	653	586	198	213	(1239)	(411)	851	799
I A 4	—	—	410	395	—	—	410	395
II A 1	118	130	—	—	—	—	118	130
II A 2	181	205	194	168	386	362	375	373
II A 3	144	129	36	36	(273)	(72)	180	165
II A 4	—	—	156	137	—	—	156	137
					(1512)	(483)		
♂ heterozygous	2588	2505	1566	1512	1508	1497	4154	4017
I B 1	285	253	—	—	—	—	285	253
I B 2	418	418	362	413	836	775	780	831
I B 3	180	164	51	66	(344)	(117)	231	230
I B 4	—	—	319	282	—	—	319	282
II B 1	91	90	—	—	—	—	91	90
II B 2	118	104	116	100	222	216	234	204
II B 3	124	107	37	40	(231)	(77)	161	147
II B 4	—	—	32	35	—	—	32	35
					(575)	(194)		
♀ heterozygous	1216	1136	917	936	1058	991	2133	2072
Grand total 3 : 1					(2087)	(677)		
Grand total (ex. 3 : 1)	3804 : 3641		2483 : 2448		2566 : 2488		6287 : 6089	

Column I refers to the groups of Table I.
Figures in brackets where 3 : 1 ratios expected; all others, 1 : 1 ratio expected.
Small letters give *phenotypes*: b, black (**BCW**); r, red (**bbCW**); bn, black, no-white (**BCww**); rn, red, no-white (**bbCww**); a, albino (**ccW**); c, colourless (**ccww**).
Columns VI and VII give the total **W** and **ww** individuals respectively; columns VIII and IX give the total **C** and **cc** individuals respectively.

The ratios derived from these figures are as follows (Table III):

TABLE III.

		Expected Dom. Rec.	Actual Dom. Rec.
(a) **C** versus **c** (cols. VIII and IX)	♂ het.	1 : 1	1·034 : 1
	♀ het.	1 : 1	1·029 : 1
	Total	1 : 1	1·033 : 1
(b) **W** versus **w** (cols. VI and VII)	♂ het.	1 : 1	1·007 : 1
	♀ het.	1 : 1	1·068 : 1
	Total	1 : 1	1·031 : 1
	♂ het.	3 : 1	3·130 : 1
	♀ het.	3 : 1	2·964 : 1
	Total	3 : 1	3·083 : 1

When the **C–c** segregation is analysed further according as it occurs in a **W** or a **ww** stock, the following figures are found:

TABLE IV.

			Expected Dom. Rec.	Actual Dom. Rec.
(c) **CW** versus **ccW** (cols. II and III)	♂ het.		1 : 1	1·033 : 1
	♀ het.		1 : 1	1·070 : 1
	Total		1 : 1	1·045 : 1
(d) **Cww** versus **ccww** (cols. IV and V)	♂ het.		1 : 1	1·036 : 1
	♀ het.		1 : 1	0·980 : 1
	Total		1 : 1	1·014 : 1

(4) *Variations in linkage.* In Fig. 1 there have been constructed curves for frequency distribution of different intensities of linkage in both male and female. The families have been selected with regard to the total numbers on which cross-over values could be determined (*i.e.* omitting all albinos and colourless). All families with 20 such individuals or less have been rejected. Superposed graphs are given for all with 21 or over, for all with 31 or over, and for all with 41 or over. The cross-over values have been grouped by classes of 5 per cent. c.o.v.—0–5 5–10, 10–15 per cent. and so on.

It will be seen that there is a great range of linkage intensity (Table V).

TABLE V.

No. of individuals on which c.o.v. determined	No. of matings ♂	No. of matings ♀	Range of cross-over value ♂ %	Range of cross-over value ♀ %	Modal class ♂ %	Modal class ♀ %
>20	67	32	5·6–59·5	31·3–75·9		
>30	53	26	5·6–59·5	31·3–68·8	20–25	50–55
>40	39	19	9·3–41·9	31·3–60·0		

The skew curve for the male is natural, since to reduce crossing-over, *e.g.* from 20 to 15 per cent., is a greater proportionate change than to do so from 40 to 35 per cent.; the female curve is somewhat skew, but in the other sense, falling rapidly after the class 50–55 per cent. This again is intelligible if 50 per cent. represents the extreme theoretical value and the actual values above this are due merely to chance. On the other hand, the fact that the mode comes throughout in the class above 50 per cent. is surprising, especially in view of the fact that all families with a value of exactly 50 per cent. are counted in the class below. This modal value of above 50 per cent. is a more striking fact than that the mean value should be above 50 per cent., since the mean is only 50·6 per cent. It is theoretically possible on the ordinary loop

theory of crossing-over to have values over 50 per cent., but no definite case has ever been recorded. On the other hand, according to Bernstein's theory (1928) it should be impossible to obtain values above 50 per cent. The skewness of the curve, however, favours the view that the female
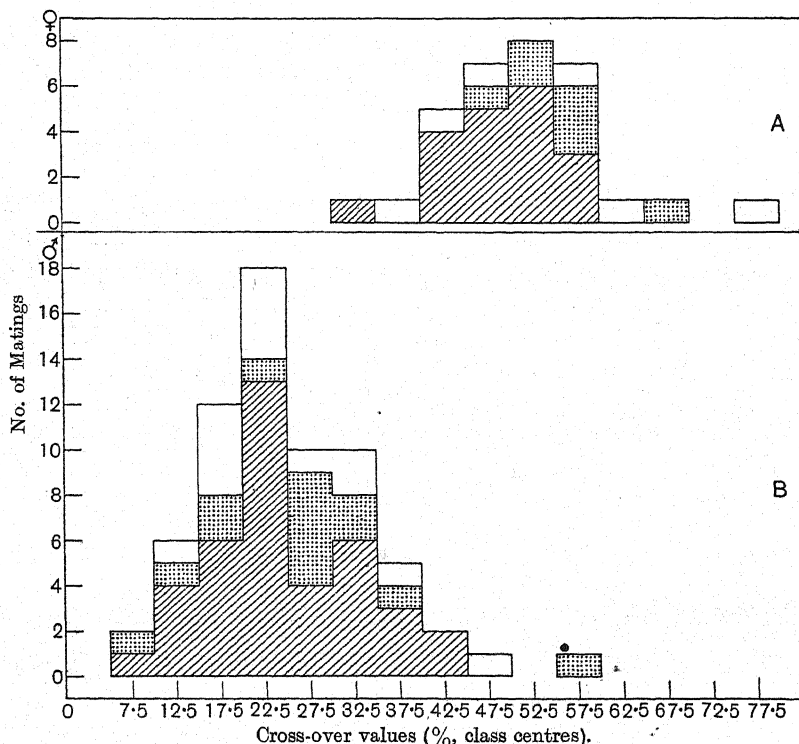
Fig. 1. Histograms for frequency of crossing-over between the loci **B** and **C** in *Gammarus chevreuxi*: *A* in females; *B* in males. The oblique-shaded areas represent the frequencies obtained when only those matings are included which gave a total of cross-overs plus non-cross-overs of over 40 individuals; the addition of the dotted areas gives the frequency for families whose corresponding totals were over 30; and the inclusion of the clear areas for those with corresponding totals over 20. Matings which gave less than 20 individuals on which cross-over parentage could be calculated are not included.

shows linkage with high c.o.v., with some variation below 50 per cent., but with 50 per cent. as the theoretical limit.

(5) *Genetic basis of cross-over variation.* Table VI shows the cross-over percentages of various lines of descent. Unfortunately it was not possible to mate males with related females, and so practise a rigorous selection for high and low cross-over values. None the less, there are clear

TABLE VI.

The names of the families are given in the centre, and their relationship indicated by lines of descent. The figures on either side give, in order, (1) non-cross-overs, (2) cross-overs, (3) total, non-cross-overs + cross-overs, (4) c.o.v. They are placed in line with the family to which they refer, on the left when the ♀ parent was heterozygous, on the right when the ♂ parent was heterozygous.

♀ (1)	♀ (2)	♀ (3)	♀ c.o.v.	Family	♂ (1)	♂ (2)	♂ (3)	♂ c.o.v.
				CPL 10	7	45	52	86·5
				⌐1 CPL 10 A				
				⌐2 CPL 10 A|A	21	8	29	27·6
46	45	91	49·6	⌐2 CPL 10 A|B				
				–3 CPL 10 A|B|A	9	1	10	10·0
				–3 CPL 10 A|B|B	36	10	46	21·7
				–3 CPL 10 A|B|C	35	10	45	22·2
				⌐1 CPL 10 B				
				⌐2 CPL 10 B|A	15	22	37	59·5
				⌐1 CPL 10 C				
				⌐2 CPL 10 C|A	74	41	115	35·7
				⌐3 CPL 10 C|A|A = CPLC 9 . .	64	9	73	12·3
5	8	13	61·5	⌐1 CPLC 9 A				
—	6	6	100·0	⌐1 CPLC 9 B				
2	4	6	66·7	⌐1 CPLC 9 D				
13	9	22	40·9	⌐1 CPLC 9 E				
5	4	9	44·4	⌐1 CPLC 9 F				
				⌐2 CPL 10 C|E	7	1	8	12·5
				⌐2 CPL 10 C|F	24	12	36	33·3
26	22	48	45·9	⌐1 CPLC 3|C				
2	6	8	75·0	⌐1 CPLC 3|D				
				⌐1 CPL 10 E				
				⌐2 CPL 10 E|C	24	6	30	20·0
				⌐1 CPL 10 H				
				⌐2 CPL 10 H|A	21	6	27	22·2
				⌐2 CPL 10 H|B	42	9	51	17·6
				⌐2 CPL 10 H|D	11	7	18	38·9
				⌐CPL 47 fr. CPL 10	4	5	9	55·6
				⌐1 CPL 47 A	73	23	96	24·0
				⌐1 CPL 47 B	31	17	48	35·5
41	28	69	40·6	⌐1 CPL 47 C				
50	55	105	52·4	⌐CPL 48 fr. CPL 10				
65	60	125	48·0	⌐CPL 55				
				⌐1 CPL 48 A	22	5	27	18·5
2	5	7	71·4	⌐1 CPL 48 B				
2	3	5	60·0	⌐1 CPL 48 C				
14	21	35	60·0	⌐CPL 53 = 1 CPL 48 D				
				⌐1 CPL 53 A	16	9	25	36·0
				⌐CPL 54	75	40	115	34·8
				⌐1 CPL 54x	38	21	59	35·6
7	10	17	58·8	⌐CPL 46				
				⌐CPL 49	70	23	93	24·7
				⌐1 CPL 49|A . . .	5	1	6	16·7
				⌐2 CPL 49|A|A				
8	8	16	50·0	⌐1 CPL 49|B				
8	5	13	38·5	⌐CPL 52				
				⌐CPL 52	23	10	33	30·3
				⌐1 CPL 54 A	48	25	73	34·2
				⌐1 CPL 54 B	2	1	3	33·3
				⌐1 CPL 54 C	10	1	11	9·1
				⌐1 CPL 54 D	11	4	15	26·7
				⌐1 CPL 54 F	8	4	12	33·3
				⌐1 CPL 54 J	16	3	19	15·8
				⌐CPLC 32	25	9	34	26·5
152	165	317	52·0	Total CPL 10 descendance (excl. CPL 10):	860	343	1203	28·5

TABLE VI (*continued*).

				CPL 24	47	13	60	21·7
7	22	29	75·8	⌐1 CPL 24 A				
49	36	85	42·4	⌐1 CPL 24 B				
				⌐2 CPL 24 B|A	62	21	83	25·3
				⌐CPLC 34	6	5	11	45·5
				⌐2 CPL 24 B|B	43	10	53	18·9
				⌐CPLC 33	22	7	29	24·1
				⌐2 CPL 24 B|C	43	12	55	21·9
25	26	51	51·0	⌐1 CPL 24 C				
				⌐2 CPL 24 C|A	47	12	59	20·3
18	19	37	51·4	⌐1 CPL 24 D				
				Total	270	80	350	22·8
				Descendance only	223	67	290	23·1
				CPLC 26	32	23	55	41·9
				⌐1 CPLC 26 A	22	18	40	45·0
				⌐1 CPLC 26 B	28	10	38	26·3
1	2	3	66·7	⌐1 CPLC 26 C				
				⌐1 CPLC 26 D				
				⌐2 CPLC 26 D|A	19	10	29	34·5
				Total	101	61	162	37·6
				Descendance only	69	38	107	35·5
24	20	44	45·5	CPL 32				
8	13	21	61·9	⌐1 CPL 32 A				
				⌐1 CPL 32 B	36	10	46	21·7
				⌐1 CPL 32 C	50	16	66	24·2
					86	26	112	23·2
				CPL 35	118	27	145	18·6
				⌐1 CPLC 35 A	12	1	13	7·7
				⌐1 CPLC 35 B	87	10	97	10·3
				⌐2 CPLC 35 B|H	46	7	53	13·2
				⌐1 CPLC 35 C	11	5	16	31·3
				⌐CPLC 3 (2nd ♂) . . .	50	31	81	38·3
				⌐1 CPLC 35 D	47	20	67	29·9
				⌐1 CPLC 35 E	36	10	46	21·7
				Total	407	111	518	21·4
				Descendance only	289	84	373	22·5
				F_1 descendance only	193	46	239	19·2
				CPLC 45	78	8	86	9·3
				⌐CPLC 45 A	46	14	60	23·3
				⌐2 CPLC 45 A|A	8	4	12	33·3
				⌐CPLC 45 B	67	30	97	30·9
				⌐CPLC 45 C	17	10	27	37·0
				⌐2 CPLC 45 C|A	14	13	27	48·2
				⌐2 CPLC 45 C|B	5	1	6	16·6
				Total	235	80	315	25·4
				Descendance only	157	72	229	31·4
14	18	32	56·3	Rep. 4				
22	33	55	60·0	⌐Rep. 4 A				
				⌐Rep. 4 B	74	34	108	31·5
				⌐2 Rep. 4 B|A	18	6	24	25·0
				⌐2 Rep. 4 B|B	13	7	20	35·0
				⌐2 Rep. 4 B|C	11	3	14	21·4
36	**51**	**87**	**58·6**		116	50	166	30·2

TABLE VI (*continued*).

				Rep. 7 a	6 33	33 6 }	39	{84·6 {15·4
3	3	6	50·0	−1 Rep. 7 a A				
				−1 Rep. 7 a B	78	18	96	18·8
				−1 Rep. 7 a C	24	6	30	20·0
				−1 Rep. 7 a D	19	12	31	38·7
26	21	47	44·7	−CPLC 47				
				−CPLC 44	13	6	19	31·6
				−CPLC 45	78	8	86	39·3
10	22	32	68·8	−1 Rep. 7 a E				
				−1 Rep. 7 a G	42	8	50	16·0
				−CPLC 40	25	7	32	21·2
				−1 Rep. 7 a H	4	1	5	20·0
17	16	33	48·5	−CPLC 28				.
56	62	118	52·5	Descendance only	283	66	349	18·9
				F_1's only	167	45	212	21·2

indications of part of the variation in crossing-over values in the male having a genetic basis. This is clearly seen on examining the figures for the totals of the lines of CPLC 26, CPL 35 and Rep. 7a with the c.o.v.'s of 37·6, 21·4 and 18·9 per cent. (descendance only) on totals of 162, 518 and 349 respectively. The small line of Rep. 4 B also has high values, and the larger line of CPL 24 (7 families) has medium-low values (22·8 per cent. on a total of 350).

Two families call for comment—CPL 10 and Rep. 7α. Both of these were chosen to found lines on account of the peculiar cross-over values found. CPL 10, if correctly reported, had 45 cross-overs on a total of 52; and Rep. 7α, 33 on a total of 39, both giving a c.o.v. of over 80 per cent. None of the descendance showed this phenomenon, and the simplest explanation is to assume that the asserted make-up of the males employed was in error, and that CPL 10 was really a case of repulsion, Rep. 7α one of coupling. This would give c.o.v.'s of 13·5 and 15·4 per cent. respectively. The descendance of CPL 10 is very variable in cross-over value.

A peculiar case is afforded by the line of CPLC 45. This male had a very low c.o.v. (9·3 per cent.) but his descendance (6 families) averaged 31·4 per cent.—a high value.

If sufficient data had been accumulated for females, it would be possible to test whether the female c.o.v. of 50 per cent. was due to true crossing-over, or to independent assortment. If the former, it

should be possible to select out low c.o.v. lines; if the latter, this should be impossible. There are not at present sufficient data to decide between these alternatives. In *Drosophila,* strains breeding true for high and low have been selected out. It is clear that the same could be done with *Gammarus.*

Four years ago (Huxley, 1924) I prophesied on the basis of the markedly lower value of crossing-over in the male that *Gammarus* would be found to be heterogametic in the male sex. This has since been cytologically shown to be the case by Palmer (1926).

It appears to be a general rule that wherever crossing-over is absent or markedly reduced in one sex, that sex is the heterogametic sex. This of course applies to the autosomes. There are no known cases of crossing-over in sex-chromosomes in the heterogametic sex save in certain Teleost fishes. The converse does not hold; the two sexes often have very similar values for crossing-over. Where the sexual difference in c.o.v. is slight, the rule also does not hold—*e.g.* Mammals.

The cases so far recorded are as follows:

(1) No crossing-over in autosomes of heterogametic sex

   (*a*) heterogametic sex male—*Drosophila,*

   (*b*) heterogametic sex female—silkworm.

(2) Crossing-over markedly reduced in heterogametic sex

   (*a*) heterogametic sex male—grasshoppers, *Paratettix, Apotettix, Gammarus,*

   (*b*) heterogametic sex female—no cases recorded.

There is a clear biological reason for the existence of this rule. We may summarise the argument as follows:

(1) It is in general an advantage to have the sex-determining difference between the male-determining and female-determining chromosome of the heterogametic sex a considerable one. If the difference is slight, it may be easily overridden by external agencies, and genetic males become somatic females and *vice versa* (as appears to occur in frogs and certain fish), leading to upsets in sex-ratio in the existing and subsequent generations.

(2) A considerable sex-determining difference is usually to be obtained only by a difference in several factors. The sole case where it would appear that only one sex-factor is involved is in the Teleosts *Aplocheilus* (Aida, 1921) and *Lebistes* (Schmidt, 1920; Winge, 1927), where crossing-over occurs between **X** and **Y**. This type of fish is in addition notoriously unstable sexually (Essenberg, 1926).

(3) Once two or more factors are involved in sex-determination, it

will be advantageous to prevent crossing-over between **X** and **Y**. If this is not done, some of the cross-over chromosomes will determine intersexes, which is clearly disadvantageous.

(4) Where the **Y** is absent, or markedly smaller than the **X**, no crossing-over in the **XY** pair can occur. This state of affairs, however, is clearly secondary, and the primitive need must have been to prevent possible crossing-over in this pair. This could have been accomplished by alteration in the **XY** pair alone, which may account for the usual peculiar behaviour of the sex-chromosomes in remaining condensed through maturation (heteropycnosis).

It could equally well be prevented by suppressing crossing-over altogether in *all* chromosomes of the heterogametic sex. Marked reduction of autosomal crossing-over would also presumably facilitate total suppression of crossing-over in the **XY** pair.

(5) As Muller (1914) pointed out, the absence of crossing-over between **X** and **Y** will lead to the **Y** being shielded from the effects of selection, and to recessive mutations accumulating in it until it becomes completely (or almost completely—Stern, 1926) "empty" genetically. The "emptiness" of the **Y** chromosome appears thus to be in the long run dependent upon the need for suppression of crossing-over in the **XY** pair, and this upon the need for a stable sex-determining mechanism; as does also the absence or marked reduction of crossing-over sometimes found in the heterogametic sex.

SUMMARY.

In *Gammarus chevreuxi* there is a marked sexual difference in linkage intensity. For the factors **B–C**, the mean cross-over value for females is 50·4 per cent. on 2133 specimens, for males is 25·4 per cent. on 4154 specimens. It is impossible at present to decide whether the state of affairs in the female is due to true linkage with high c.o.v., or to independent chromosomes which undergo pseudo-linkage in the male; though the former is the more probable.

There is great variation in linkage intensity in both sexes; but the shape of the frequency polygons obtained is different for the two sexes. The variation appears to be due in large part to genetic causes.

The fact that absence or marked reduction of crossing-over in the autosomes of one sex has only so far been recorded for the heterogametic sex is mentioned and its theoretical bearings discussed.

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POLYMORPHISM IN THE MOTH *ACALLA COMARIANA* ZELLER.

By J. C. F. FRYER, M.A.

(With One Plate.)

CONTENTS.

THE Tortricid moth *Acalla (Peronea) comariana* is a member of a group of species, formerly included in the comprehensive genus *Peronea*, of which several are conspicuously polymorphic both in wing colour and pattern, two species of the group—*A. hastiana* L. and *A. cristana* F.—being generally regarded as the most variable of all Lepidoptera, an Order itself remarkable for colour variability. In spite of this feature, no genetical studies appear to have been made with any member of the Peroneid group, possibly because of the difficulty of dealing with the better known species in captivity. When, therefore, in 1924 material of *A. comariana* became available, the opportunity was taken of making some preliminary experiments which showed that with this species no insuperable difficulties need be anticipated either in pairing the adults or in rearing their progeny. Definite breeding experiments were therefore started, and soon gave results which suggested that the scheme of inheritance of the different colour forms could be explained along relatively simple Mendelian lines. In the subsequent experiments this anticipation has in part been fulfilled, but it has also become apparent that within the major colour forms there is considerable minor variation which has not yet been explored, while in addition it has been discovered that the species has separated into local races differing from each other both in regard to the proportion in which the major forms are represented and in regard also to the colour tone of similar forms. Also there is some evidence of the occurrence of an insect indistinguishable from *comariana*

but with a different life-history and affecting a different food-plant. It is thus evident that some years of work may be necessary to render the study of *comariana* at all complete, and it therefore seems advisable to put on record the facts so far gathered.

Finally, it may be mentioned that the investigation has been essentially a "spare time" employment which has often had to give way to more pressing claims. Under these circumstances the author's thanks are specially due to those who have assisted him, notably to Professor Punnett in regard to the Mendelian interpretation of the results, to Messrs Buckhurst and Spickernell of the Ministry of Agriculture for material of the insect from Wisbech, to Mr Mansbridge for a supply of the Lancashire race, to Mr Sheldon for dealing with the nomenclature of the different forms(1), and finally to Mr B. S. Williams of the Plant Pathological Laboratory, on whom has fallen most of the practical work involved in the experiments.

The Colour Forms of *Acalla comariana*.

Acalla comariana is a member of the Tortricidae—a section of the Tortricina. It appears in literature under various genera—notably *Peronea, Oxygrapha* and *Acalla*—but as the present paper is not concerned with the classification of the Tortricidae, the merits of these various generic appellations need not be discussed.

In the Tortricid forewing the pattern most widely found has as its chief features: (*a*) a fascia passing from near the centre of the costa obliquely to the tornal angle of the wing, and (*b*) a sub-triangular or semicircular blotch placed more or less centrally on the costa with the base of the triangle (or diameter of the half circle) coinciding with the costal margin. The fascia thus includes a considerable part, and often all, of the costal blotch, but except where the generalised pattern is altogether displaced, both costal blotch and fascia remain as distinct entities, sometimes coloured in strong contrast to each other and the remainder of the wing, and sometimes merely outlined by scales of different colour or density. The fascia and costal blotch occupy a considerable proportion of the surface of the wing, but there is always left a basal area between the inner edge of the fascia and the thoracic articulation, and a terminal area between the outer edge of the fascia and the wing margin. These areas also have typical markings (notably a short dorsal line or dash in the basal area) but often they give the impression of indicating the "ground colour" of the wing upon which the fascia and blotch and other markings have been superimposed.

This simple, and probably primitive, pattern is obscured or replaced in certain species or forms of the Peroneid group but it has been largely retained in *A. comariana*, as will be seen from the following descriptions of the seven major forms which are known.

Proteana Hg. (Pl. II, fig. 1). Apart from the costal blotch, the whole wing including the fascia is a shade of grey, while the costal blotch is chestnut-brown. The inner edge of the fascia is faintly indicated by tawny scales and the outer edge also, but even less distinctly. The minor variations within the form consist *inter alia* in the tone of grey, which may be "cold" when the majority of the scales are grey, or "warm" when the grey scales are freely mixed with others of a fuscous brown or yellow. The costal blotch may be darker or lighter chestnut, while in its centre near the costa is a lighter area, often yellowish in colour, and at the apex of the blotch another yellowish suffusion.

Potentillana Cooke (Pl. II, fig. 2). The whole wing is grey as in *proteana* but the costal blotch is black. The fascia is outlined rather indistinctly with tawny as in *proteana*. In regard to minor variations, there is a variable light area in the costal blotch as in *proteana*, and the ground colour shows the same range of variation except that it may become even browner. This is most notable in the Lancashire race and will be referred to in the following section.

Latifasciana Sheldon (Pl. II, fig. 3). The wing, apart from the fascia and costal blotch, is white thickly spotted with black scales, giving a sort of pepper-and-salt appearance. The costal blotch and fascia are chestnut-brown, the edge of the costal blotch being hardly indicated. Minor variation concerns the extent to which black and white predominate in the ground colour, some specimens having the black and white areas about equal in extent, while others have the black very largely predominating. The extension of the chestnut-brown fascia outwards towards the margin of the wing is also variable: in some specimens it almost reaches the margin so that practically no "ground colour" is visible.

Fasciana Sheldon (Pl. II, fig. 4). The wing is as in *latifasciana* except that the costal blotch is black and as a rule black scales occur rather more frequently throughout the whole wing, so that even chestnut-brown areas appear colder in tone. Minor variations follow the same lines as in *latifasciana*.

Brunneana Sheldon (Pl. II, fig. 5 *A*). In the typical form the whole wing is brown, the costal blotch being a darker brown than the remainder, though there is an evident light centre to the blotch in most specimens.

The fascia edges are also indicated in scales of the darker brown shade. There is, however, much variation within the form and it is not improbable that one of these variations may require a special name. In this latter variation (Pl. II, fig. 5 *B*) the whole of the fascia is of a slightly darker brown shade than the base of the wing, which latter in some specimens becomes lighter as it nears the inner edge of the fascia. The costal blotch, although detectable, is merged in the fascia, and some specimens have a slight resemblance to a *latifasciana*, although they are perfectly distinct owing to the fact that there is no black speckling. A further variation of the *brunneana* form, which is most evident in the Lancashire race, consists in the suppression of practically all major markings so that the whole wing is a suffused brown. It is probable that one of these two sub-forms of *brunneana* was used by Zeller in describing the species, but as the experiments indicate that the former has a definite genetic constitution whereas nothing is yet known as to the latter, it would be unwise to use the name *comariana* for any form at present.

Comparana Sheldon (Pl. II, fig. 6). The wing is brown except for the costal blotch and certain minor markings, which are black. The fascia edge is very faintly indicated in darker brown scales or may not be discoverable at all. The insect has a general resemblance to the species *Acalla comparana* Hb. There is not much variation in this form but the tone of the ground colour may be less "warm" and tend towards a more ochreous brown, while there may be indications of a brown dash in the centre of the costal blotch.

Fuscana Sheldon (Pl. II, fig. 7 [1]). The wing is fuscous brown, varying somewhat in shade: the typical markings are much obscured but in most specimens can be detected, and this remark applies specially to the fascia, of which both margins are usually indicated by yellowish-brown scales. The costal blotch is practically of the same fuscous brown as the remainder of the wing, and no *fuscana* forms with either a black or brown costal blotch have yet been observed.

RACES IN *A. COMARIANA*.

The breeding experiments discussed subsequently were all made with *comariana* obtained in the Wisbech district where the species frequents strawberry, to which it is sometimes a serious pest: it is double-brooded and has not *there* been found on any wild food-plant. The major forms

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[1] Owing to the enlargement Fig. 7, although accurate, makes the insect appear too light in colour. The impression given by an average *fuscana* is that of an almost black moth.

which occur are *proteana, potentillana, latifasciana, fasciana,* and *comparana. Fuscana* is not known from the district. Collections of larvae have been made in Wisbech strawberry fields in 1926 and 1927 and the moths reared with the results are given in the following table:

Form	1926 A		1926 B		1927 A		1927 B		Collections %
	Totals	%	Totals	%	Totals	%	Totals	%	
Proteana	48	27·9	14	41	24	32·4	13	42	31·8
Potentillana	17	10	—	—	6	8·1	1	3·2	7·7
Latifasciana	32	18·5	4	12	11	15	5	16·1	16·7
Fasciana	3	1·7	—	—	1	1·4	—	—	1·3
Brunneana	24	14	7	21	9	12	5	16·1	14·5
Comparana	48	27·9	9	26	23	31·1	7	22·6	28

1926 *A* and *B* two separate fields, June brood.
1927 *A*, June brood, 1927 *B*, August-September brood, different fields.

The sample 1926 *B*, comprising 34 specimens, is probably too small to reckon as a separate entity, and the same remark applies to 1927 *B*, but even so the distribution of the six forms in the four samples is remarkably similar and suggests that the mean gives a fair approximation to the distribution of the major forms in the Wisbech district.

The second race of *comariana*, now being brought into the experiments, comes from Lancashire, where it feeds upon *Comarum palustre* and is said to be single-brooded. As, however, Lancashire larvae feed equally well upon cultivated strawberry and damage to strawberry in Lancashire by the species has been reported, no specialisation in regard to food-plant is involved, while the single-brooded habit is probably climatic in origin and not fixed. Two samples of larvae from the same district in Lancashire have been reared and the moths classified, the figures for 1926 having been kindly supplied by Mr Sheldon and the larvae for 1927 by Mr Mansbridge:

Form	1926		1927		Mean %
	Totals	%	Totals	%	
Proteana	16	15·3	47	18·5	17·6
Potentillana	42	40	111	44	42·8
Brunneana	8	7·6	11	4·3	5·3
Comparana	2	1·9	7	2·8	2·5
Fuscana	37	35·2	77	30·4	31·8

So far as these samples are concerned, therefore, the Lancashire race of *comariana* differs from the Wisbech not only in the presence of another major form (*fuscana*) but also in the absence of two major forms (*latifasciana* and *fasciana*) and in the distribution of the remainder: *potentillana,* for instance, a relatively scarce form in Wisbech, is most abundant of all in the Lancashire individuals, while *comparana,* one of the most common forms in Wisbech, is quite rare in Lancashire.

As a whole, the colour of the Lancashire forms tends to be dull as compared with similar forms of the Wisbech race. Specially noteworthy are uniform brown forms of *brunneana* with the costal blotch hardly showing and forms of *potentillana* (presumably) in which brown scales are freely mixed with grey, causing the wing, apart from the costal blotch, to appear as brownish grey or even tan-coloured. Any discussion of such forms is, however, premature and they are merely mentioned to complete the description contrasting the Lancashire and Wisbech races.

Finally, reference may be made to what is possibly a third race, unless it proves to be a distinct species. This "race" is distinguished first by the fact that it feeds upon greenhouse azaleas, and secondly in that it spends the winter as a larva, whereas *comariana* does so as an egg. The insect is known on the Continent of Europe as *A. comparana* Hb., but a single larva found on imported *Azalea indica* at Harpenden early in 1926 produced on May 2nd a moth ($\male$) which was indistinguishable from *Acalla comariana* form *proteana*, and on dissection proved to have the genitalia of that species and not of *A. comparana* Hb. Subsequently a second moth was obtained by Mr Miles, of Manchester University, in an azalea house in Belgium, and this again proved to be indistinguishable morphologically from *A. comariana* and had none of the characteristics of *A. comparana*. The evidence in favour of an azalea race of *comariana* is thus not yet great, but the facts are sufficiently interesting and suggestive to justify mention.

TECHNIQUE.

The life-history of the Wisbech *comariana* has been described by Petherbridge(2) but some account of its habits in captivity and the methods found best for dealing with it may be of use to others wishing to work with the insect or its near allies. A calendar of the species is roughly as follows: October–March, egg; April–mid-June, larva and pupa; mid-June–mid-July, moth, egg; mid-July–August, larva and pupa; August–September, moth, egg.

At first attempts were made to imitate as nearly as possible the conditions in which the insects normally live, and both adults and larvae were provided with strawberry plants growing in pots and kept under cages. Little success was thus obtained, and after trials in different directions the most simple methods were found the best, and the insects are now dealt with as follows:

The male and female which it is desired to pair are placed in a circular glass-topped tin box (3 in. in diameter × 1 in. in depth). Inside

the tin, on the bottom, is a filter paper to absorb excess moisture, and a strawberry leaf partly for the female to lay eggs upon and partly to keep the atmosphere from becoming too dry. In about 57 per cent. of the pairs thus confined, fertile eggs are laid, beginning about the third day and continuing for 10 days to a fortnight[1]. The eggs are laid some on the leaf, some on the glass of the lid, and some on the sides of the tin—which seems to have greater attraction for the female than anything else, an unfortunate preference as the over-wintering eggs frequently die if laid on the tin, and this in spite of careful insulation from rapid temperature changes. In the summer brood, however, eggs laid on the tin hatch normally. Never in captivity has a female laid winter eggs on strawberry stipules, the site found by Petherbridge to be selected in the open. In the case of winter eggs, hatching takes place from the beginning of April onwards, while the summer eggs hatch about ten days after they are laid. It is noteworthy that fertile winter eggs turn brick red, while fertile summer eggs remain green but occasionally a tendency on the part of the summer brood to become single-brooded has been noted in that a few eggs have turned red. Considerable development in such eggs has been observed, but they have never hatched either in the summer or the following spring.

On hatching, whether in spring or summer, the young larvae are allowed to remain in the tin and are fed upon strawberry leaves, to which they are moved by means of a brush. Thence, throughout their larval stages, the insects are handled as little as possible, fresh leaves being merely added and mouldy ones, which have been deserted, removed. Pupation takes place between two leaves spun together or in a fold in a leaf. The pupae are then carefully removed and placed singly in corked glass tubes (about $1\frac{1}{2}$ in. long), being allowed to lie on the bottom of the tube without any covering. The label with the brood number is placed inside the tube so that the moth, on emergence, may have a surface sufficiently rough to enable it to crawl up and dry its wings. With this system remarkably few pupae fail to emerge satisfactorily, while the tubes offer a great advantage in recording the form and sex of the moth, and finally indiscriminate mating, which might occur if several insects emerged simultaneously in one vessel, is prevented.

The method adopted is thus exceedingly simple, and reduces the labour of handling many broods—at best, considerable—to a minimum. The chief criticism which may be made is that the broods are small—

[1] Female *comariana* lay eggs occasionally even when unmated, but such eggs have never produced larvae and no evidence of parthenogenesis has yet been obtained.

averaging fourteen moths per brood, but it is not known to what this is due. No method of feeding the adults has been found, and although they live for a fortnight or so it is possible that, if fed, they might lay more eggs. Finally, it may be noted that it is important to record the adult moths while quite fresh, for as soon as they get worn or faded it may be exceedingly difficult to detect the forms, in spite of the great differences between them.

<div align="center">BREEDING RESULTS.</div>

As was pointed out in a paper on breeding *Papilio polytes* (3), it is seldom possible in working with butterflies and moths to make only those pairings which are required, for in the first place the emergence of any one brood is spread over a period which is lengthy in relation to the effective life of the individual moths, and secondly little over 50 per cent. of fertile matings can be counted upon. It follows, therefore, that pairings must often be made with such moths as are available, merely in order to ensure the continuance of the experiments, and this explains why many of the broods subsequently referred to are not derived from the ideal matings from the theoretical standpoint.

A table showing the results of the different matings is given on p. 177. Each brood appears under a brood number, and the fact that these numbers are not consecutive is explained by the failure of the matings made under the missing numbers. The origin of the parents is indicated by the brood number: thus, "♀ *proteana* 4 × ♂ *potentillana* 3" implies that the ♀ was from "brood 4" and the ♂ from "brood 3." Where after the brood number of the ♂ a second higher figure appears in brackets thus (in brood 32) "♂ *proteana* 4 (27)," it means that the male was used twice and was the parent both of broods 27 and 32. At the beginning of the experiments, and occasionally subsequently, it was necessary to introduce individuals reared from wild collected Wisbech larvae, and they are indicated by the letter "W" in place of a brood number.

From the description of the major forms given previously, it is evident that apart from the costal blotch there are three "ground colours"—*i.e.* the grey of *proteana* and *potentillana*, the "marbled" or "brindled" of *latifasciana* and *fasciana*, and the brown of *brunneana* and *comparana*. An examination of the table of results will show:

(1) That grey × grey never gives anything but grey—*e.g.* broods 8, 27, 35, 36, 51, 60, 61, 70, 85, 119, 124.

(2) That marbled × marbled gives marbled—*e.g.* broods 25, 69, 71, 107, or grey and marbled—*e.g.* broods 42, 55, 57, 103.

(3) That brown × brown gives brown and marbled (brood 111), brown and grey (broods 49, 58, 90, 104), and finally brown alone (brood 108), although this last brood is too small to be of much value.

As regards matings between the different forms, it appears that:

(4) Marbled × grey gives marbled (broods 34, 115) or grey and marbled (broods 3, 4, 41, 45, 73, 113, 114, 120).

(5) Brown × grey gives brown (broods 88, 123), brown and grey (broods 2, 32, 40, 44, etc.), and brown and marbled (brood 197).

(6) Brown × marbled gives brown (brood 56—but only a very small brood), brown and marbled and grey (broods 53, 62, 89), or brown and grey (broods 77, 106).

From this it appears that grey is recessive to both brown and marbled, and that marbled is recessive to brown.

Next, as regards costal blotch, the table shows:

(1) That brown × brown gives only brown (broods 9, 42, 53, etc.). (Two broods appear to contradict this assertion—Nos. 34 and 116—in each of which a single individual with a black costal blotch appeared in families in which otherwise the costal blotch was brown. It is believed, however, on the evidence of the experiments as a whole that the appearance of these two individuals was due to a slip in handling the broods, very possibly by the introduction of a "wild" egg or minute larva on the food-plant.)

(2) That brown × black gives black (broods 80, 87, 114, 120, 125) and brown and black (2, 32, 47, etc.).

(3) That black × black gives black (broods 70, 104, etc.) and brown and black (broods 3, 32, 62, etc.).

The black costal blotch, therefore, appears to be dominant over the brown.

EXPLANATION OF RESULTS.

In seeking a theoretical explanation based on this analysis, an attempt was first made to explain the inheritance of ground colour on a "presence and absence" basis—treating "grey" as being produced in the absence of factors for brown and marbled, but some of the observations are not consistent with such an hypothesis and a better explanation is obtained by regarding the brown, marbled and grey ground colours as being due to three factors in a series of multiple allelomorphs, the factor for brown ground colour being dominant over that for marbled and the latter over that for grey.

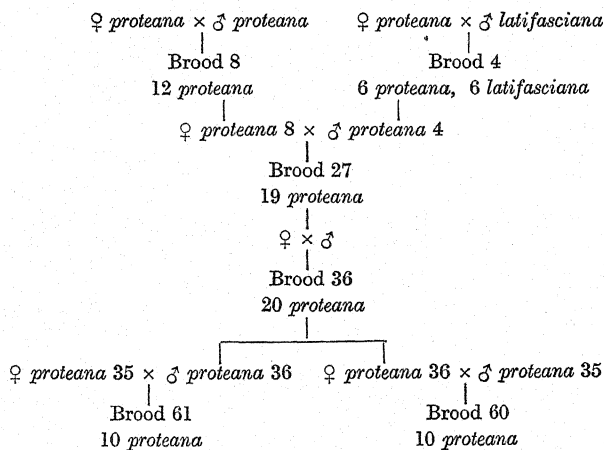If this hypothesis be applied to the results, it will be found adequate

provided that no attention be paid to the distribution of the two colours of costal blotch within the several families, but when this latter point is examined, it is evident that something further is required to supply an explanation of many of the observations. For instance, in brood 47, a ♀ *proteana* (grey ground colour, brown costal blotch) was paired with a ♂ *comparana* (brown ground colour and black costal blotch) and resulted in 7 *proteana* and 10 *comparana* instead of the four forms *proteana, potentillana, brunneana* and *comparana,* as might have been expected since the ♂ parent was evidently heterozygous. In order to explain this and similar observations, it is necessary to introduce into the hypothesis the suggestion that the factors for costal blotch are closely linked with those for ground colour: an individual, therefore, which is heterozygous both for blotch and ground colour will thus produce only, or mainly, two types of gamete instead of four. Finally, it may be mentioned that in the results so far obtained, there is nothing to suggest that there is any relation between sex and coloration.

The scheme, therefore, at present adopted as a working hypothesis is (1) that the ground colours are due to a series of three multiple allelomorphs, B (brown), B^1 (marbled) and B^2 (grey), (2) that black costal blotch is due to a factor C in the absence of which (c) the blotch is brown, and (3) that there is close linkage between costal blotch colour and ground colour, all the factors being in a pair of autosomes.
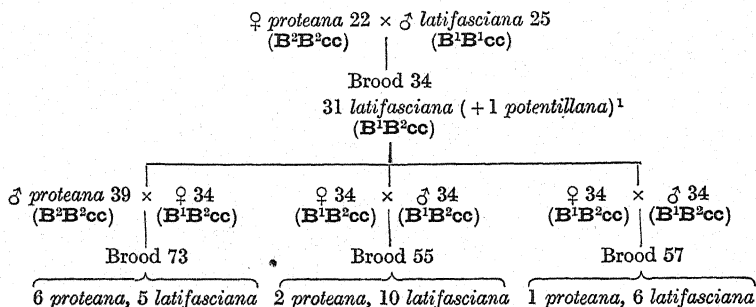
According to the above hypothesis, the different major forms of *comariana* will be expressed by the following formulae:

1. *Proteana* (grey ground colour, brown costal blotch) $= B^2B^2cc$
2. *Potentillana* (grey ground colour, black costal blotch)
                                  $= B^2B^2Cc, B^2B^2CC$
3. *Latifasciana* (marbled ground colour, brown costal blotch)
                                  $= B^1B^1cc, B^1B^2cc$
4. *Fasciana* (marbled ground colour, black costal blotch)
               $= B^1B^1Cc, B^1B^2Cc, B^1B^2CC, B^1B^1CC$
5. *Brunneana* (brown ground colour, brown costal blotch)
                                $= BB^1cc, BBcc, BB^2cc$
6. *Comparana* (brown ground colour, black costal blotch)
            $= BBCC, BBCc, BB^1CC, BB^1Cc, BB^2CC, BB^2Cc$

(1) In applying these formulae to the experimental results it hardly seems necessary to trace in any detail matings in which the double recessive *proteana* alone was concerned, but as an instance the reader is referred to the broods given in the following pedigree:
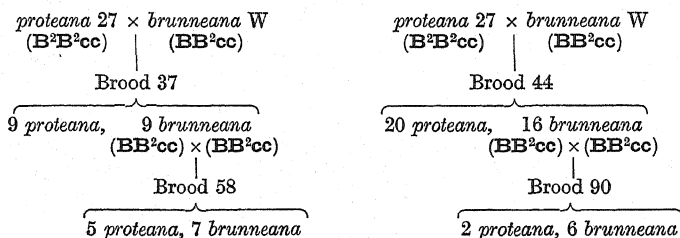
♀ *proteana* × ♂ *proteana*   ♀ *proteana* × ♂ *latifasciana*
| |
Brood 8   Brood 4
12 *proteana*   6 *proteana*, 6 *latifasciana*
| |
♀ *proteana* 8 × ♂ *proteana* 4
|
Brood 27
19 *proteana*
|
♀ × ♂
|
Brood 36
20 *proteana*
|

♀ *proteana* 35 × ♂ *proteana* 36   ♀ *proteana* 36 × ♂ *proteana* 35
| |
Brood 61   Brood 60
10 *proteana*   10 *proteana*

(2) As regards the relationship between *proteana* and *latifasciana*, attention is drawn to broods 34, 55, 57 and 73:

♀ *proteana* 22 × ♂ *latifasciana* 25
(B^2B^2cc)   (B^1B^1cc)
|
Brood 34
31 *latifasciana* (+1 *potentillana*)[1]
(B^1B^2cc)
|

♂ *proteana* 39 × ♀ 34   ♀ 34 × ♂ 34   ♀ 34 × ♂ 34
(B^2B^2cc) | (B^1B^2cc)   (B^1B^2cc) | (B^1B^2cc)   (B^1B^2cc) | (B^1B^2cc)

Brood 73   Brood 55   Brood 57

6 *proteana*, 5 *latifasciana*   2 *proteana*, 10 *latifasciana*   1 *proteana*, 6 *latifasciana*

It may be pointed out that the ♂ parent of brood 34 was obtained from a *latifasciana* (B^1B^2cc) × a *fasciana* (B^1B^2Cc) and it might therefore have been B^1B^1cc or B^1B^2cc, the result showing that it was actually of the former constitution, since when paired with the double recessive *proteana*, only *latifasciana* were produced. These *latifasciana* should all have been of the constitution B^1B^2cc, and therefore when mated with *proteana* should have given equal numbers of *proteana* and *latifasciana*, as was shown by brood 73. When mated together *latifasciana* of 34 should have given *proteana* and *latifasciana* in the 1 : 3 proportion, but in the experiments the proportion given was 2 : 10 in brood 55 and 1 : 6 in brood 57, there being thus a small excess of *latifasciana*.

[1] The recording of a single *potentillana* in brood 34 is assumed to have been due to a technical error, such as the introduction of a minute wild larva on the food-plant.
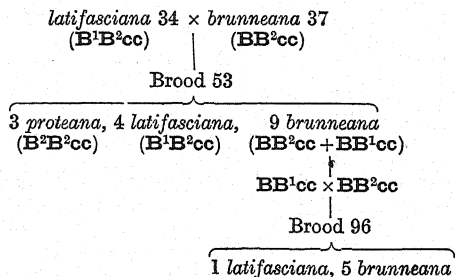
(3) The relationship between *proteana* and *brunneana* may be illustrated by broods 37, 44, 58 and 90:

proteana 27 × *brunneana* W
(B^2B^2cc) | (BB^2cc)

Brood 37

9 *proteana*,   9 *brunneana*
(BB^2cc) × (BB^2cc)

Brood 58

5 *proteana*, 7 *brunneana*

proteana 27 × *brunneana* W
(B^2B^2cc) | (BB^2cc)

Brood 44

20 *proteana*,   16 *brunneana*
(BB^2cc) × (BB^2cc)

Brood 90

2 *proteana*, 6 *brunneana*

In each of these pedigrees the original ♂ parent was bred from a wild larva and its constitution was assumed to be BB^2cc. The results are in accordance with the supposition except that in brood 58 the *proteana* individuals are in excess.

(4) The relationship between *brunneana* and *latifasciana* may be instanced by broods 34, 37, 53, 96.

The pedigrees of broods 34 and 37 are shown in the two previous sections. A ♀ *latifasciana* from 34, having the constitution B^1B^2cc, was mated with a ♂ *brunneana* of 37 (BB^2cc) and the resulting broods were as follows:

latifasciana 34 × *brunneana* 37
(B^1B^2cc) | (BB^2cc)

Brood 53

3 *proteana*, 4 *latifasciana*,   9 *brunneana*
(B^2B^2cc)   (B^1B^2cc)   ($BB^2cc + BB^1cc$)

$BB^1cc × BB^2cc$

Brood 96
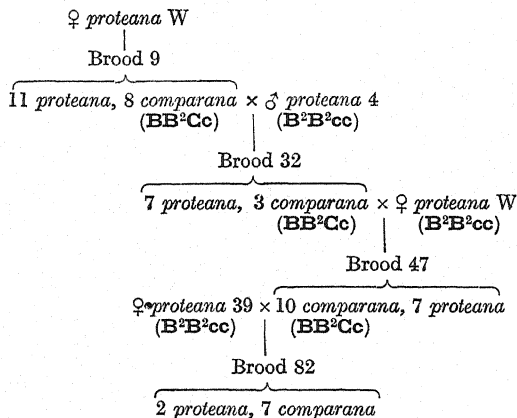
1 *latifasciana*, 5 *brunneana*

Brood 53, containing *proteana*, *latifasciana* and *brunneana* in the proportions 1 : 1 : 2, is in accordance with expectations: brood 96 is unfortunately small but it is also approximately in accordance with the hypothesis since *brunneana* and *latifasciana* in the proportion 3 : 1 was expected. (So far as these results alone are concerned the parents of 96 might also have been $BB^1cc × BB^1cc$ but subsequent broods, notably 111 and 121, show that one of them was carrying grey.)

Finally it may be mentioned that later in the experiments it was observed that certain *brunneana* individuals (Pl. II, fig. 5 *B*) differed from the typical form in the more feeble differentiation of the costal blotch and the greater development of the fascia—as has been described

previously (p. 162). Such forms occurred in broods 53, 63, 66, 77, 89, 96, 111 and 116, and it can hardly be a coincidence that in each of these broods, and in these broods only, individuals of the constitution BB^1cc should have occurred according to the hypothesis: it is therefore believed that these "fasciate" *brunneana* are heterozygous for B and B^1. The belief is borne out by the fact that some of the fasciate *brunneana* obtained in later broods are more brilliantly coloured, and these have a definite resemblance to *latifasciana*. Experiments are now in progress to test this assumption.

(5) The pedigrees so far given deal only with ground colour inheritance without the complication of the costal blotch. The hypothesis provides that the factor for costal blotch be linked with that for ground colour, and since five factors in all are concerned, the number of possible combinations is considerable. First may be considered a case where dominant costal blotch (black) is linked with a dominant ground colour:

$$\begin{array}{c}
\text{♀ } proteana \text{ W} \\
| \\
\text{Brood 9}
\end{array}$$

11 *proteana*, 8 *comparana* × ♂ *proteana* 4
   (BB^2Cc)   (B^2B^2cc)

Brood 32

7 *proteana*, 3 *comparana* × ♀ *proteana* W
   (BB^2Cc)   (B^2B^2cc)

Brood 47

♀ *proteana* 39 × 10 *comparana*, 7 *proteana*
   (B^2B^2cc)   (BB^2Cc)

Brood 82

2 *proteana*, 7 *comparana*

From the results of brood 9 it is assumed that the ♂ parent must have been a *comparana* (brown ground colour, black costal blotch) of the constitution BB^2Cc. If there had been no linkage, or if C had been linked with B^2, *potentillana* forms (grey ground colour, black costal blotch) must have appeared in the brood, which is of sufficient size to be representative. All brood 9 *comparana*, therefore, must also have been BB^2Cc, and if mated with a *proteana* would be expected to give only these forms again in the brood—as happened in 32, although *proteana* forms were in excess. A ♂ of 32—again BB^2Cc—was next mated with a wild bred *proteana* ♀ to give brood 47, which consisted of 7 *proteana* and 10 *comparana*, and finally one of the latter was mated with a *proteana*

of 39, giving 2 *proteana* and 7 *comparana* in brood 82. These five pairings (including that giving brood 9) must all have been $\mathbf{BB^2Cc} \times \mathbf{B^2B^2cc}$ $= \mathbf{BB^2Cc} + \mathbf{B^2B^2cc}$, and there appears to have been no breaking in the linkage.

In the experiments in only one other series of families is the black costal blotch linked with a dominant ground colour. This series begins with brood 97, which was produced by a wild bred *comparana* ♀ mated with a ♂ *potentillana* (grey ground colour, black costal blotch) of 81. This *potentillana* was the result of a cross between a *brunneana* ♀ by a *potentillana* ♂ and it must therefore have had the constitution $\mathbf{B^2B^2Cc}$. Brood 97 was as follows:.

♀ *comparana* W × ♂ *potentillana* 81
$(\mathbf{BB^1Cc})$ | $(\mathbf{B^2B^2Cc})$

Brood 97

6 *latifasciana*, 8 *fasciana*, 11 *comparana*
♀ × ♂

Brood 104

5 *potentillana*, 9 *comparana*

The presence of *fasciana* and *latifasciana* in brood 97 shows that the *comparana* parent carried $\mathbf{B^1}$, while the presence of *latifasciana* shows that $\mathbf{B^1}$ must have been linked with $\mathbf{c}$. The *comparana* parent therefore must have been $\mathbf{BB^1Cc}$. Since the ♂ *potentillana* can only have been $\mathbf{B^2B^2Cc}$, the constitution of the brood should have been as follows:

$$\mathbf{BB^1Cc} \times \mathbf{B^2B^2Cc} = \mathbf{B^1B^2cc} + \mathbf{B^1B^2Cc} + \mathbf{BB^2CC} + \mathbf{BB^2Cc}$$

the numbers of the *comparana* being theoretically equal to the sum of the other two forms, although actually the latter are in slight excess. According to the hypothesis, *comparana* of brood 97 could not carry $\mathbf{B^1}$ but must carry $\mathbf{B^2}$, and this is proved by brood 104, as shown in the pedigree. The parents of brood 104 might both have had the constitution $\mathbf{BB^2CC}$, or one of them might have been $\mathbf{BB^2Cc}$, the latter alternative being the case, as demonstrated by subsequent experiments (see brood 113). The linkage between ground colour and costal blotch in these two broods also appears very close, since if there had been any crossing-over *brunneana* ($\mathbf{BB^2cc}$) should have appeared in brood 97.

As regards the factor for "marbled" ground colour $\mathbf{B^1}$, no case has yet been observed where it is linked with $\mathbf{C}$, the factor for the dominant costal blotch, but $\mathbf{B^1}$ has only twice been brought into the experiments from the wild source—first with the male parent of broods 3 and 4, and

secondly with the female parent of 111, so the chance of detecting the combination B^1C has not been great.

Linkage between the factors for one of the two dominant ground colours and that for the recessive costal blotch occurs frequently throughout the experiments and this is equally true of the opposite condition in which the factor for the dominant costal blotch is linked with that for the recessive ground colour (grey). Heterozygous individuals of *fasciana* will thus have the constitution B^1B^2Cc, and those of *comparana* BB^2Cc. In this connection two types of mating are of special interest:

(1) BB^2cc (or B^1B^2cc) $\times B^2B^2Cc$.

(2) BB^2Cc (or B^1B^2Cc) $\times B^2B^2cc$.

Matings of type 1 should give in the family four forms of individuals in equal numbers, while those of type 2 should give only two forms, which forms should be different from either of the parents in that the recessive costal blotch would have passed to the forms with the dominant ground colour and *vice versa*.

Of matings of type 1 there are a considerable number, which may be brought together in the following table:

Brood	Parent	Proteana (B^2B^2cc)	Potentillana (B^2B^2Cc)	Brunneana (BB^2cc) or latifasciana (B^1B^2cc)	Comparana (BB^2Cc) or fasciana (B^1B^2Cc)
68	$BB^2cc \times B^2B^2Cc$	1	4	—	5
76	$B^2B^2Cc \times BB^2cc$	—	4	2	5
78	$BB^2cc \times B^2B^2Cc$	—	1	6	1
81	$B^2B^2Cc \times BB^2cc$	1	7	2	8
84	$B^2B^2Cc \times BB^2cc$	4	3	6	2
121	$BB^2cc \times B^2B^2Cc$	2	4	—	3
3	$B^2B^2Cc \times B^1B^2cc$	2	7	6	13
113	$B^2B^2Cc \times B^1B^2cc$	5	6	3	4
117	$B^1B^2cc \times B^2B^2Cc$	2	1	4	2
	Total	17	37	29	43

The broods are in most cases small and the occasional absence of one of the forms anticipated out of four is not surprising, but such errors should be eliminated if sufficient broods are considered together. However, even by taking nine similar broods comprising 126 individuals, nothing like parity is obtained between the different forms, and it is possible that some point of importance has been missed. It would not seem that the result can be due to crossing-over for the forms with the black costal blotch are in marked predominance (80) over those with brown (46), in spite of the fact that of the four types of gamete taking part in any mating only one carried the factor C assumed responsible for the black blotch.

As regards matings of type 2 ($\mathbf{BB^2Cc}$ or $\mathbf{B^1B^2Cc} \times \mathbf{B^2B^2cc}$), only one (brood 114) occurs in the experiments, and as it is of some importance, the pedigree may be given.

$$\text{♀ } \textit{fasciana } 97 \times \text{♂ } \textit{latifasciana } 97$$
$$(\mathbf{B^1B^2Cc}) \qquad (\mathbf{B^1B^2cc})$$

Brood 107

6 *latifasciana*,	2 *fasciana*
($\mathbf{B^1B^1cc} + \mathbf{B^1B^2cc}$)	($\mathbf{B^1B^2Cc}$)

$$\text{♀} \times \text{♂ } \textit{proteana } \mathrm{W}$$
$$(\mathbf{B^1B^2Cc}) \qquad (\mathbf{B^2B^2cc})$$

Brood 114

10 *potentillana*,	5 *latifasciana*
($\mathbf{B^2B^2Cc}$)	($\mathbf{B^1B^2cc}$)
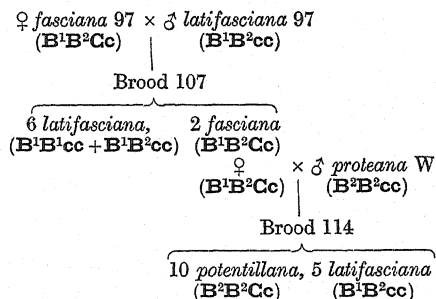
The pedigree of brood 97 is given on p. 170 and the constitution of the *fasciana* forms both of brood 97 and of brood 107 is evidently $\mathbf{B^1B^2Cc}$.[1] All *proteana* are of constitution $\mathbf{B^2B^2cc}$ and therefore the parents of brood 114 were $\mathbf{B^1B^2Cc} \times \mathbf{B^2B^2cc}$ and the matings should have resulted in equal numbers of *potentillana* ($\mathbf{B^2B^2Cc}$) and *latifasciana* ($\mathbf{B^1B^2cc}$). The family in fact contains only these forms although *potentillana* was in considerable excess. The assumed linkage between the factors for ground colour and costal blotch is, however, entirely borne out, since any crossing-over would have produced both *proteana* and *fasciana*[2].

General Conclusions.

The broods and pedigrees cited as examples in the previous section comply reasonably well with the demands of the hypothesis from the qualitative point of view; those forms which are expected occur in the

[1] Brood 107 should theoretically have contained also *potentillana* forms equal in number to the *fasciana*.

[2] [Note added July, 1928. Since the above account was written, further results have become available, and it seems desirable to quote additional cases of the matings $\mathbf{BB^2Cc}$ or $\mathbf{B^1B^2Cc} \times \mathbf{B^2B^2cc}$ in which the dominant factors $\mathbf{B}$ or $\mathbf{B^1}$ are linked with the recessive factor $\mathbf{c}$ for costal blotch colour. These are as follows:

Brood			Potentillana	Latifasciana	Brunneana
131	♀ *comparana* 121	× ♂ *proteana* 124	6	—	9
132	♀ *proteana* 113	× ♂ *fasciana* 120	9	10	—
137	♀ *fasciana* 113	× ♂ *proteana* 119	5	5	—
144	♀ *comparana* 123	× ♂ *proteana* 124	10	—	10
147	♀ *proteana* 124	× ♂ *comparana* 123	5	—	9
168	♀ *proteana* 113	× ♂ *comparana* 125	10	—	8

In each of these matings the results are in accordance with those expected, and there appears to have been no breaking in the linkage which is assumed to exist between the factors for ground colour and costal blotch.]

great majority of the families and where there are absences they are in relatively small broods in which four different forms should appear and in which in consequence an occasional absence may reasonably be attributed to chance. These remarks apply equally to those families which have not been discussed in detail. In three broods, however, forms appear which should not do so in accordance with the hypothesis. In two of these broods—34 and 116—the "wrong" form occurs as a single individual only, and may with reasonable certainty be attributed to a technical error, such as the chance introduction of a young egg or larva with the food-plant or a defective tin (occasionally the glass in the lid is slightly loose so that a newly-hatched larva can escape). The third case, however, that of brood 2, is in a different category. Here a pairing recorded as *comparana* ♀ × *proteana* ♂ gave 3 *proteana*, 1 *potentillana*, 7 *brunneana* and 13 *comparana*—a result which appears difficult to explain. It is possible that the parents had the constitution **BB²Cc** and **B²B²cc** and that the linkage between the factors for ground colour and those for costal blotch largely broke down, but in view of the absence of evidence of "crossing-over" in the remainder of the experiments, this explanation is not convincing and in the writer's opinion it is more probable that some technical error was responsible, especially as the family occurred at the beginning of the experiments when methods of handling the insects were still under trial. Unfortunately every mating made with individuals of brood 2 proved fruitless, and its peculiarities were not further explored.

Apart from this brood, the chief discrepancy between the hypothesis and the results consists in the proportions in which the different forms occur in certain families, and notably in those of type **B²B²Cc** × **BB²cc** listed on p. 171. In a majority of cases, however, the numbers in which the forms occur are as near to the hypothetical as could be expected in the case of small broods, and on the whole the inheritance of the several forms used in these experiments appears to be reasonably in accord with the hypothesis suggested.

This statement receives further support in the subsequent paragraphs in which the results are explored from a rather different point of view.

The great similarity of the various samples drawn from the Wisbech population, referred to on p. 161, suggests that these samples indicate approximately the numerical distribution of the major forms in the Wisbech district. The mean figures of the four samples were as follows: *proteana* 31·8 per cent.; *potentillana* 7·7 per cent.; *latifasciana* 16·7 per cent.; *fasciana* 1·3 per cent.; *brunneana* 14·5 per cent.; *comparana*

28 per cent. Without pressing the accuracy of these figures it may safely be concluded that *proteana* and *comparana* are the predominant forms and that *fasciana* is relatively very scarce. Such predominance or scarcity might be due to a number of causes, among which may be mentioned (1) some external selective agency which has favoured the more numerous forms and *vice versa*, (2) a factor of the "lethal" type resulting in the destruction of certain forms in the pre-imaginal stages, (3) some form of selective "infertility" whereby the matings required to produce the less numerous forms are relatively infertile, and (4) the "age" of the different forms—the less numerous being the newcomers which have not yet had time to assume their proper numerical proportions in the population. Any full discussion of these different possibilities would perhaps occupy more space than the data now available would warrant, but so far as the experiments bear upon them some brief reference seems desirable.

Of the different hypotheses just advanced:

(1) The experiments cannot be expected to throw light on the possible existence of external selective agencies in nature, but if such agencies in fact exist, it is difficult to believe that they react directly through the medium of the different wing patterns. Reference to the figures representing *latifasciana* and *fasciana* will show that the difference between them is small and consists essentially in the colour of the costal blotch. It would hardly seem reasonable that when the form with the brown costal blotch exists to the extent of 16·7 per cent. of the population, the imposition of a black costal blotch would so hamper its possessors that they could exist only to the extent of 1·3 per cent., and this in spite of the fact that in another "pair" of forms, *comparana* and *brunneana*, it is the form with the black costal blotch which has the advantage. Again it is difficult to imagine a form of selection which at Wisbech would reduce *potentillana* to the proportion of 7·7 per cent. of the population while allowing the same form to become predominant in Lancashire and to occupy 42·8 per cent. of the population. There is therefore a definite improbability in an hypothesis formulating the existence of an external selective agency to account for the facts observed.

(2) Next as to the second possibility, it would seem that evidence of the existence of a factor of a "lethal" type might be found in the manner in which the experimental results deviate from those required by the hypothesis for explaining the inheritance of the different forms: thus, if taking the experiments as a whole one form proved to be markedly

deficient in numbers the existence of a lethal factor might be suspected. In order to follow up this reasoning the numbers of the different forms demanded by the hypothesis were calculated for all broods in which two or more forms should have appeared and the sums of these hypothetical figures are compared with the actual results in the following table:

	Proteana	Poten-tillana	Lati-fasciana	Fasciana	Brun-neana	Com-parana
Actual figures	180	130	141	49	131	195
Hypothetical figures	189	134	142·5	43·75	131·75	185
Actual figures as percentages	21·8	15·7	. 17·1	6	15·8	23·6
Hypothetical figures as percentages	22·9	16·2	17·3	5·3	15·9	22·4

The numbers of the different forms expected and those actually found thus differ relatively little and afford no suggestion of the existence of any differential mortality in the pre-imaginal stages, and the comparison is thus chiefly of interest in that it tends to support the hypothesis explaining the inheritance of the different forms.

(3) The third possibility that there may be some form of selective infertility affecting the proportion of the different forms in nature cannot be satisfactorily tested without more data than are actually available, but such evidence as there is lends no support to this theory. For instance selective infertility might either cause a high proportion of sterile matings between those individuals destined to produce the less numerous forms, or it might reduce the size of the families obtained from such matings. If anything of the kind were occurring, some evidence of it might be expected in matings and families in which the scarce form *fasciana* was concerned. As regards the first alternative, fifteen attempts were made to obtain matings which should have resulted in broods containing *fasciana* forms. Of these matings six failed and nine produced fertile ova—*i.e.* 60 per cent. were successful, a figure which compares very well with that of 57, the percentage of successful matings in the experiments as a whole. As to the second alternative, there were nine broods containing the *fasciana* form, the average size of the family being 16·2, which figure is the same as that of the average brood produced by eleven matings of the double recessive *proteana* (the predominant form in the Wisbech population) and exceeds the average number in the family for the experiments as a whole. There would, therefore, seem no reason for suspecting the presence of any factor operating against matings producing *fasciana* forms, and it is unlikely that an explanation of the scarcity of this form will be found in such a direction.

(4) Of the four hypotheses, therefore, there remains but the last—that the proportions in which the different forms now occur are an indication of their age in the particular population under consideration. Fisher(4) argues that in the absence of selection, the time required for a single mutation to affect one-third of the genes of the species will be of the order of that required for a number of generations equal to the total population of the species. This, he suggests, would postulate a far longer period of time than can usually be allowed. The subject is evidently one which must be left to the statisticians, since the mere layman is only too likely to fall into error. The only point to which it is proposed to draw attention is that in the Wisbech population, the B^2C combination seems to be decidedly scarce and that the existence of B^1C has not yet been demonstrated. It is therefore possible that the combination B^2C is relatively recent in the Wisbech population, and that B^1C may not yet even exist—the *fasciana* only being produced by the combination of B^1c with B^2C.

Finally, it may be suggested that in dealing statistically with insects it is worth remembering first, that the total population varies from period to period within extraordinarily wide limits, and secondly that certain species are very sedentary and so tend to break up into small groups or populations, individuals of one group but rarely passing into the next. Thus, the Wisbech population of *comariana* in a time of "depression" is certainly not more than 1 per cent. of its numbers during more favourable periods, indeed it is probably very much less. At the same time *comariana* is a relatively sedentary species, so that the interchange between the Wisbech population and other populations will be small. It would thus seem permissible to treat such a population as a definite entity, while its total numbers would not necessarily be so immense as might at first sight appear. These remarks are made in the hope that they may prompt criticisms and suggestions from the statistical point of view in order that in the further work now in progress points of importance may not be overlooked.

EXPERIMENTAL DATA.

The number after each parent denotes the brood in which it arose;
W signifies a wild individual.

Brood	♀ Parentage	♂ Parentage	Pro-teana	Poten-tillana	Lati-fasciana	Fasciana	Brun-neana	Com-parana
1	Proteana	× ?	3	—	—	—	1	13
2	Comparana	× proteana	3	1	—	—	7	13
3	Potentillana	× latifasciana	2	7	6	13	—	—
4	Proteana	× latifasciana (3)	6	—	6	—	—	—
8	Proteana	× proteana	12	—	—	—	—	—
9	Proteana	× ?	11.	—	—	—	—	8
22	Proteana 4	× potentillana 3	4	2	—	—	—	—
25	Latifasciana 4	× fasciana 3	—	—	7	3	—	—
27	Proteana 8	× proteana 4	19	—	—	—	—	—
32	Comparana 9	× proteana 4 (27)	7	—	—	—	—	3
33	Proteana 27	× proteana 27	2	—	—	—	—	—
34	Proteana 22	× latifasciana 25	—	(1)	31	—	—	—
35	Proteana 22	× proteana 22	36	—	—	—	—	—
36	Proteana 27	× proteana 27	20	—	—	—	—	—
37	Proteana 27	× brunneana W	9	—	—	—	9	—
39	Proteana 32	× proteana 27	25	—	—	—	—	23
40	Potentillana 22	× comparana 32	5	7	—	—	—	—
41	Potentillana 22	× fasciana 25	—	14	8	6	—	—
42	Latifasciana 25	× latifasciana 25	9	—	34	—	—	—
44	Proteana 27	× brunneana W	20	—	—	—	16	—
45	Proteana 32	× latifasciana 25	18	—	10	—	—	10
47	Proteana W	× comparana 32	7	—	—	—	—	1
49	Comparana W	× comparana 32 (47)	—	1	—	—	2	—
51	Proteana 40	× potentillana 40	2	6	—	—	—	—
53	Latifasciana 34	× brunneana 37	3	—	4	—	9	—
55	Latifasciana 34	× latifasciana 34	2	—	10	—	—	—
56	Latifasciana 34	× comparana 40	—	—	—	—	—	5
57	Latifasciana 34	× latifasciana 34	1	—	6	—	—	—
58	Brunneana 37	× brunneana 37	5	—	—	—	7	—
60	Proteana 36	× proteana 35	10	—	—	—	—	—
61	Proteana 35	× proteana 36	10	—	—	—	—	2
62	Latifasciana 34	× comparana 40	2	—	5	—	2	—
63	Latifasciana 34	× brunneana 37	—	—	—	—	—	—
65	Proteana 37	× proteana 37	12	4	2	—	4	5
66	Brunneana 44	× fasciana 41	—	4	—	—	—	5
68	Brunneana 37	× potentillana 41	1	4	3	—	—	—
69	Latifasciana 34	× latifasciana 34	—	—	3	—	—	—
70	Potentillana 41	× potentillana 41	—	7	—	—	—	—
71	Latifasciana 42	× latifasciana 42	—	—	5♀	—	—	—
73	Latifasciana 34	× proteana 39	6	—	5	—	2	5
76	Potentillana 41	× brunneana 44	—	4	—	—	3	3
77	Brunneana 44	× latifasciana 41	—	1	—	—	6	1
78	Brunneana 44	× potentillana 41	—	1	—	—	—	9
80	Potentillana 41	× brunneana 44	—	10	—	—	—	8
81	Potentillana 41	× brunneana 44	1	7	—	—	2	7
82	Proteana 39	× comparana 47	2	—	—	—	—	6
83	Comparana 47	× proteana 45	5	—	—	—	—	2
84	Potentillana 49	× brunneana 44	4	3	—	—	6	—
85	Potentillana 41	× proteana 44	10	6	—	—	—	7
87	Brunneana 44	× potentillana 41 (70)	—	4	—	—	—	3
88	Proteana 44	× comparana 49	—	—	—	—	2	—
89	Brunneana 49	× latifasciana 45	6	—	3	—	7	—
90	Brunneana 44	× brunneana 44	2	—	—	—	6	—
96	Brunneana 53	× brunneana 53	—	—	1	—	5	—

Experimental Data (*continued*).

Brood	Parentage ♀		Parentage ♂	Pro-teana	Poten-tillana	Lati-fasciana	Fasciana	Brun-neana	Com-parana
97	Comparana W	×	potentillana 81	—	—	6	8	—	11
103	Latifasciana 97	×	fasciana 97	—	1	2	—	—	—
104	Comparana 97	×	comparana 97	—	5	—	—	—	9
106	Fasciana 97	×	comparana 97	—	3	—	—	—	3
107	Fasciana 97	×	latifasciana 97	—	—	6	2	—	—
108	Comparana 97	×	comparana 97	—	—	—	—	—	3
111	Brunneana 96	×	brunneana 96	—	—	5	—	10	—
113	Potentillana 104	×	latifasciana 111	5	6	3	4	—	—
114	Proteana W	×	fasciana 107	—	10	5	—	—	—
115	Latifasciana 107	×	potentillana 103	—	—	5	7	—	—
116	Brunneana 111	×	brunneana 111	—	(1)	3	—	7	—
117	Latifasciana 111	×	potentillana 104	2	1	4	2	—	—
119	Proteana W	×	proteana W	18	—	—	—	—	—
120	Latifasciana 107	×	potentillana 106	—	7	—	4	—	—
121	Brunneana 111	×	potentillana 104	2	4	—	—	—	3
122	Proteana W	×	proteana W	3	—	—	—	—	—
123	Brunneana 111	×	potentillana 104 (121)	—	—	—	—	18	16
124	Proteana W	×	proteana W	15	—	—	—	—	—
125	Brunneana 111	×	potentillana 106	—	15	—	—	—	24

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(3) Fryer, J. C. F. "An Investigation by Pedigree Breeding into the Polymorphism of *Papilio polytes* Linn." *Royal Society Philosophical Transactions*, Series B, CCIV, 227–254.

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EXPLANATION OF PLATE II.

Acalla comariana Zell.; all figs. about 2½ times natural size.

Fig. 1. *Proteana* Hg.   Fig. 2. *Potentillana* Cooke.   Fig. 3. *Latifasciana* Sheldon.
Fig. 4. *Fasciana* Sheldon.   Fig. 5 A. *Brunneana* Sheldon.   Fig. 5 B. *Brunneana* variety.
Fig. 6. *Camparana* Sheldon.   Fig. 7. *Fuscana* Sheldon.
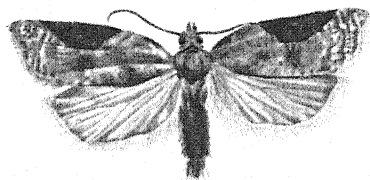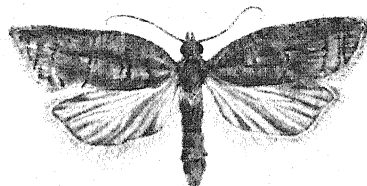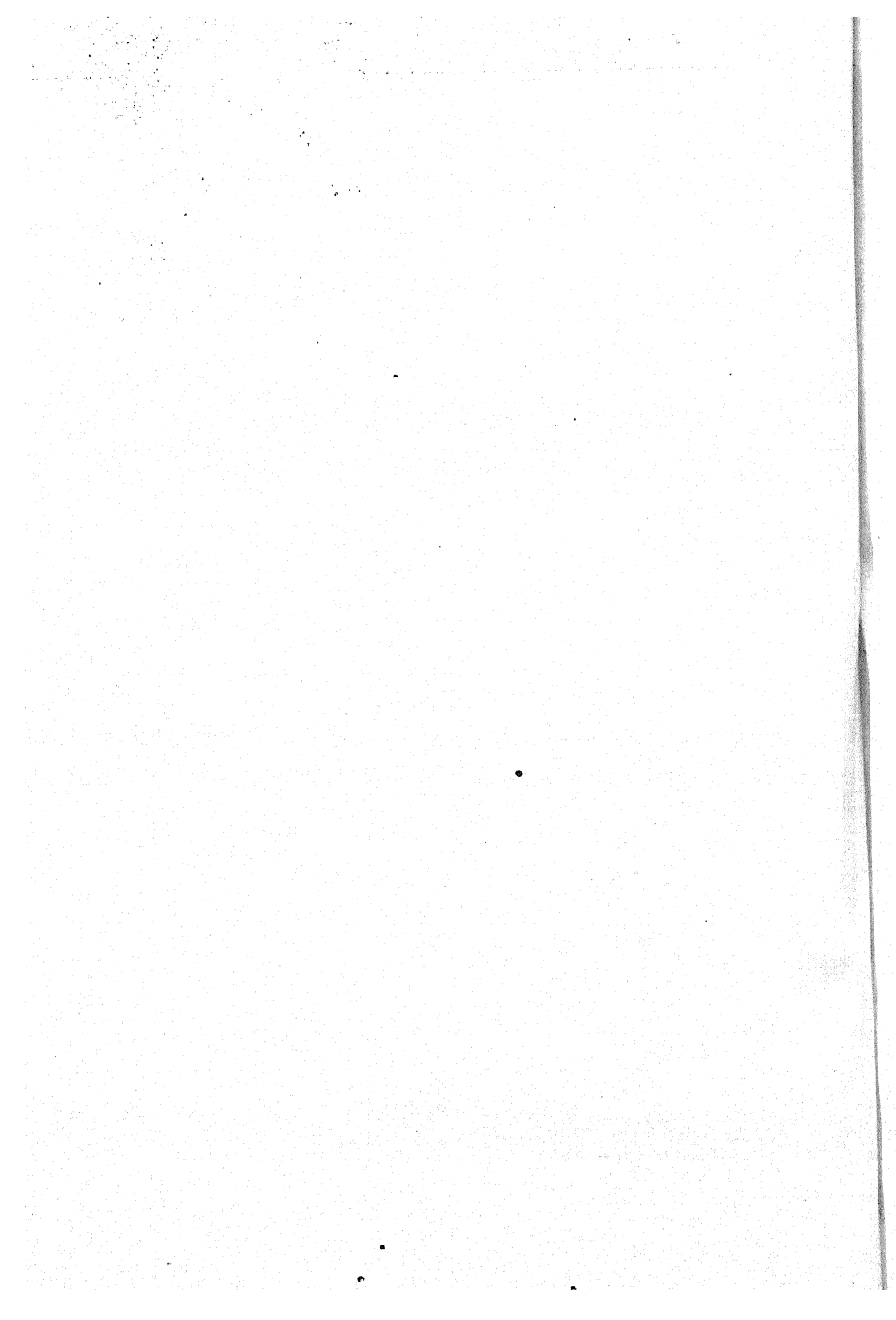
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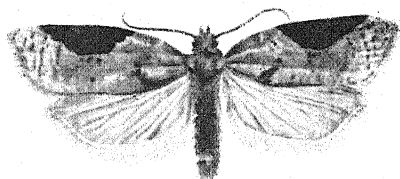
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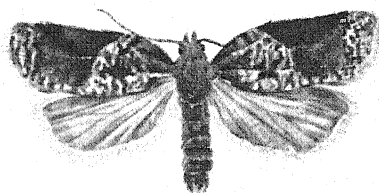
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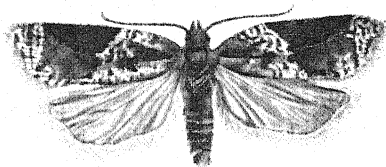
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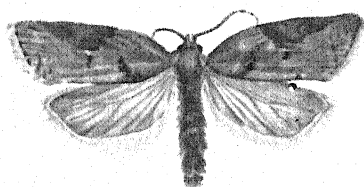
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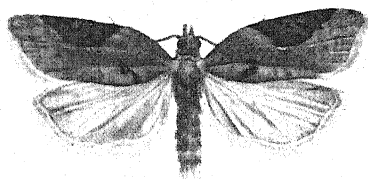
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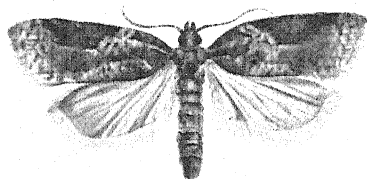
5 B

7

A CASE OF LATERAL ASYMMETRY IN THE DOMESTIC FOWL.

By F. A. E. CREW.

(With One Plate.)

A FOWL with an unusual crazy-quilt distribution of red pigment in its white plumage and with the right leg, yellow in colour, so much shorter than its fellow, the colour of which was white, that the bird not only looked lop-sided but was forced to adopt a most peculiar gait (cf. Pl. III, figs. 1 and 2), was sent by Mr Marcus Slade, a well-known breeder, to Professor Punnett, who, knowing of my interest in abnormal fowls, handed it over to me for examination. I wish to express my thanks for his generosity.

The bird was an adult F_1 individual from a Light Sussex ♀ × Rhode Island Red ♂ mating. It should have been, therefore,

either a *male*, and silver, splashed perhaps with chestnut and bay, with black pigment on neck, wings, and tail, and with white or yellow epidermal pigment according to whether the Light Sussex mother was duplex or simplex for the dominant factor for white.	or else a *female*, and gold (the exact shade being determined by the genetic constitution of the Rhode Island Red father), with black pigment on the neck, wings and tail, and with white or yellow epidermal pigment.

The colour of the Rhode Island Red is based upon a complex of several factors. In addition to the sex-linked recessive gold, there are autosomal dominants, chestnut (Agar, 1924) and bay (Hays, 1926). Gold is recessive to its allelomorph, silver, but the F_1 of this cross commonly has red markings on its saddle and elsewhere. Individuals pigmented as was this bird, though rare, are not unknown among the progeny of this mating, the pigmentation being due to the expression of the autosomal-borne factors for colour. The white epidermal pigmentation is dominant, and yellow the alternative recessive. It follows, therefore, that the Light Sussex parent, with white epidermal pigmentation, may have been either homozygous or heterozygous for this character. Had she been heterozygous, then among the progeny of this mating there would have been equal numbers of whites and yellows.

This bird was peculiar in that its characterisation was neither one nor the other of these alternatives. Its head furnishings and plumage structure were those of the male. Its attitudes and responses towards other birds were masculine, but its deformity was too severe a handicap

12-2

to allow it to fight with other males, or to play its part efficiently in sexual congress. When kept apart from males it crowed and courted hens, but during the period of observation it failed to fertilise a single egg. Because it was cock-like in plumage colour (silver) and structure, and because its sexual behaviour was typically male, it was regarded as an abnormal cock.

But since the two sides of the body were, on palpation, manifestly of different size, it was necessary to consider the possibility that the bird was a lateral gynandromorph, with the larger left side male in genetic constitution and the smaller right side female. Had it been a sex-mosaic of this kind due to the elimination from an **XX** (male) zygote of a daughter **X**-chromosome carrying the factors for either the maternal or paternal sex-linked character, and if in the fowl, as in certain other forms, the simplex and duplex states of the **X**-chromosome are associated with significant differences in the size of the body and of local parts, then the following differences in the characterisation of the two sides would be expected:

Larger side	Smaller side
Male in organisation.	Female in organisation.
XX in sex-chromosome constitution.	**X** in sex-chromosome constitution.
Silver (perhaps splashed with chestnut and bay).	Silver or gold, according to whether it was the paternal or the maternal **X** which had been lost.
Testis.	Ovary, unless the morphological and physiological destiny of the gonad is not determined by the genetic constitution of the tissue of its origin, or unless the gonads of both sides have a common origin, the cells wandering from the site of this to the position of the gonads within the body, when both gonads should have been testes or else ovaries.

The structure of the plumage on both sides would be determined by the nature of the gonads. If both were testes, then the plumage would be cocky; if one was a testis, the other an ovary, or ovotestis, then it would be henny; if both were ovaries, then it would be henny. The epidermal pigmentation would be either white or yellow, but both sides would be alike.

On superficial examination there was therefore no compelling reason to hold the view that this bird was not a gynandromorph, but the fact that its plumage was entirely cocky indicated that there was no appreciable amount of ovarian tissue present in its body. In many ways it was very similar to the abnormal fowl described by Macklin (1923) as a gynandromorph. Miss Macklin had the opportunity of examining the skeleton, head, feet, wing-tips, and gonads of a fowl which during life had been henny feathered, with the exception that in the neck-hackle there were feathers suggestive of those of the male, and that the tail-sickles were slightly longer than those of the normal hen. Its head

furnishings and sexual behaviour were male-like. It was never heard to crow, and did not fight. No abnormality in gait was noticed during life. It was killed because it was suspected of laying small eggs, and whilst being prepared for table it was noted that the right side of the body was much larger than the left, that its beak was curved to the left, and that the right side of head, comb, tongue and brain and the right orbit and nostril were larger than their fellows of the opposite side. Miss Macklin regarded this specimen as an instance of gynandromorphism due either to the elimination of an X-chromosome during the first cleavage division of a male zygote or to the synchronous fertilisation by two spermatozoa of a binucleated ovum, or of an ovum and its polar body. It was the association of a lateral difference in size comparable to the size differences beween male and female, together with the presence of a testis on the larger side and ovarian tissue on the smaller, that demanded this interpretation.

As the examination proceded, it was seen that the bird now being described had its own contribution to make to our knowledge of abnormality in the fowl. After being under observation for a year, it was killed, and the following data were secured:

		Rhode Island Red	Light Sussex
Comb	4 cm. at highest point. No indications of lateral asymmetry	Larger in ♂ (4 cm. is a good average)	Larger in ♂ (4 cm. is a good average)
Wattles	4 × 4 cm.	Do.	Do.
Eye colour	Both red	Red	Orange
Ear lobe colour	Do.	Do.	Red
Beak colour	White with black streaks. No laterality in colour distribution	Yellow	White-horn
Leg colour	Right, yellow; left, pinky-white	Do.	White
Leg scales	Those on left leg appreciably larger than those on the right		
Toe number	Four, both feet	Four	Four
Spurs	Right, 2 cm. long, horizontal, but laterally curved inwards, yellow near the base. Left, 2 cm. long, horizontal, straight, white	In ♂♂ yellow	In ♂♂ white
Gonads	A testis on either side. Left, 5·5 × 2·7 cm.; 15·735 grm. Right, 4·7 × 2·9 cm.; 13·635 grm. The difference in weight between the two testes is, in the experience of the writer, greater than that which distinguishes the testes of a normal cock. In both, there were plentiful motile spermatozoa of normal appearance. No evidence of the existence of ovarian tissue was encountered		

		Rhode Island Red	Light Sussex
Vasa deferentia	Left, larger and better developed than right; at caudal end twice as large in diameter. No oviduct		
Endocrine glands	Pituitary, thymus, thyroids, parathyroids, and adrenals were unremarkable macroscopically and histologically		
Weight	8 lbs.	♂ 9 lbs., ♀ 7	♂ 8½ lbs., ♀ 6½

When the skin and muscles had been removed, it was seen that, as in Macklin's case, the two sides of the body were markedly different in size; each bone on the left and the left half of every fused bone being, with certain exceptions, larger than its fellow on the opposite side (cf. Pl. III, fig. 3). The measurements of the bones of this bird, of Macklin's, and of the average of 22 normal males and 22 normal females, are given in the following table. I am indebted to Mr F. B. Hutt for the figures relating to these controls.

It is seen that the difference in the lengths of the bones of the two sides of the body is similar to that distinguishing the bones of male and female, and that, with the exception of ulna and tibia, the proportional lengths of the bones of this fowl are those which obtain in the case of the normal male and female.

It is seen further that this bird differs from that of Macklin in the following respects:

(1) It was the left and not the right side that was the larger.

(2) The lateral asymmetry did not affect the skull, comb, brain, and vertebral column.

(3) Sex-linked characters were involved in the mating which produced this bird.

(4) There were two testes and not one testis and an ovo-testis, and the left testis was significantly larger than the right.

It has already been argued that the presence of dissimilar gonadic tissues on the two sides of the body does not necessarily indicate that the individual is a gynandromorph. In cases of gynandromorphism in *Drosophila* the gonads are of similar structure as often as they are of dissimilar. But in the fowl there is certain evidence which points to the conclusion that genetically female tissue cannot proceed to complete spermatogenesis. It is true that this view denies the reality of the observations of several investigators, including myself, who have described active functional spermatogenesis in a reputed genetic female. It is, however, based on critical experimentation, for Domm (1927) found no evidence of any development of spermatic tissue from the germinal

TABLE I.

	Left A (cm.)	Right B (cm.)	C (cm.)	D (cm.)	Right A (cm.)	Left B (cm.)	Ratio Right/Left A	Ratio Left/Right B	Ratio ♀D/♂C	% Left>Right A	% Right>Left B	% ♂C ♀D	Rel. Left A	Rel. Right B	Rel. C ♂♂	Rel. D ♀♀	Rel. Right A	Rel. Left B
Humerus	9·70	10·5	8·206	7·303	8·10	9·0	0·835	0·86	0·889	19·75	16·66	12·37	100·00	100·00	100·00	100·00	100·00	100·00
Radius	8·80	9·8	7·369	6·513	7·95	8·3	0·903	0·85	0·884	10·69	18·07	13·14	90·72	93·33	89·80	89·92	98·15	90·22
Ulna	10·70	10·3	8·179	7·224	8·80	9·0	0·822	0·87	0·883	21·59	14·44	13·22	101·03	98·10	99·67	98·92	108·64	100·00
Carpo-metacarpus	5·40	—	4·452	3·964	5·20	—	0·963	—	0·890	3·85	—	12·31	55·67	—	54·25	54·28	64·19	—
End of wing	—	9·7	—	—	—	8·0	—	0·82	—	—	21·25	—	—	—	—	—	—	—
Femur	10·75	12·0	9·219	8·319	7·2	10·3	0·669	0·86	0·902	49·30	16·50	10·82	100·00	100·00	100·00	100·00	100·00	100·00
Tibia	16·50	16·9	13·416	11·795	13·55	13·8	0·821	0·82	0·879	21·77	22·47	13·74	153·49	140·83	145·51	141·79	188·19	133·98
Fibula	11·40	—	—	—	9·90	—	0·868	—	—	15·15	—	—	106·05	—	—	95·96	137·50	—
Tarso-metatarsus	—	22·0	9·255	7·983	—	19·0	—	0·86	0·862	—	15·79	15·92	—	—	100·39	—	—	—
Foot	10·70	10·2	—	—	9·30	9·1	0·869	0·89	—	15·05	12·09	—	—	—	—	—	—	—
Scapula	9·40	—	—	—	8·80	—	0·936	—	—	6·82	—	—	—	—	—	—	—	—
Coracoid	7·54	8·0	—	—	6·20	6·8	0·827	0·85	—	21·13	17·65	—	—	—	—	—	—	—
Clavicle	8·50	9·3	—	—	6·90	7·5	—	0·80	—	—	24·00	—	—	—	—	—	—	—
Hyoid	4·00	—	—	—	3·50	—	—	—	—	—	—	—	—	—	—	—	—	—
Mandible	7·20	—	—	—	7·10	—	—	—	—	—	—	—	—	—	—	—	—	—
Ribs; Vertebral 1	6·40	—	—	—	5·45	—	—	—	—	—	—	—	—	—	—	—	—	—
2	5·90	—	—	—	5·30	—	—	—	—	—	—	—	—	—	—	—	—	—
3	5·70	—	—	—	5·40	—	—	—	—	—	—	—	—	—	—	—	—	—
4	5·50	—	—	—	5·45	—	—	—	—	—	—	—	—	—	—	—	—	—
5	5·40	—	—	—	5·35	—	—	—	—	—	—	—	—	—	—	—	—	—
6	4·60	—	—	—	4·50	—	—	—	—	—	—	—	—	—	—	—	—	—
7	3·70	—	—	—	3·60	—	—	—	—	—	—	—	—	—	—	—	—	—
Sternal 1	5·2	—	—	—	5·1	—	—	—	—	—	—	—	—	—	—	—	—	—
2	5·7	—	—	—	4·9	—	—	—	—	—	—	—	—	—	—	—	—	—
3	5·1	—	—	—	4·4	—	—	—	—	—	—	—	—	—	—	—	—	—
4	4·2	—	—	—	3·8	—	—	—	—	—	—	—	—	—	—	—	—	—
5	3·1	—	—	—	2·9	—	—	—	—	—	—	—	—	—	—	—	—	—

A = this bird; B = Macklin's gynandromorph; C = average (both sides of body) of 22 adult males of various light breeds (Leghorn mainly); D = average (both sides of body) of 22 adult females of various light breeds.

epithelium of the ovary in a large series of experimental fowls, and he and also Lillie (1927) are of the opinion that it is exceedingly doubtful whether any genetically female fowl can ever become possessed of functional spermatic tissue. If this view is correct, then the reputed cases of sex-reversal in the fowl must be provided with another interpretation, and Macklin's case is indeed a gynandromorph. It was not necessarily a gynandromorph because the two sides of the body were of different size, for it is established that in other forms abnormality in autosomal chromosome number, as well as polyploidy and haploidy, are associated with constant and characteristic differences in body size.

In the case of the bird now described, the fact that there were two testes removed it from the class of gynandromorphs if Domm and Lillie's contention is correct. But disregarding this, the presence of spermatic tissue only does not prove that the bird was or was not a gynandromorph; nor does the fact that the two sides of the body were of male and female proportions, since in other forms aberrations in the number of autosomes is as potent a factor in the production of unusual body size as is aberration in the number of sex-chromosomes.

The fact that sex-linked characters were involved in this case might have decided this question if the maternal **X**-chromosome with its factor for silver had been the one to be eliminated, for then one side of the body would have been male and silver, the other female and gold. It follows then that either it was male and the paternal **X** was the one to be eliminated, or else the bird was not a gynandromorph.

But the laterality of the distribution of the autosomal characters, white and yellow epidermal pigmentation, permits one to suggest a concrete explanation of the peculiar characterisation of this fowl. It will be remembered that the larger side was associated with white epidermal pigmentation, the smaller with yellow. If during the early cleavage divisions of a male (genetically a larger bird than the female) zygote, heterozygous for white and yellow, the autosome carrying the factor for white was eliminated, then the parts exhibiting epidermal pigmentation, having their origin in this autosomally-deficient cell, would be yellow. If the loss of this autosome results in a genotype corresponding to smaller size, then all parts having their origin in this cell would be smaller than their fellows of the opposite side which have arisen from the sister cell of normal chromatin content. The observed fact that the skull, brain, and vertebral column were not asymmetrical can be explained on the assumption that the loss of this particular chromosome involves no loss of genes or of gene-balance affecting the

development and differentiation of these parts. If this is a reasonable explanation of the size abnormalities of this bird, and granting that Macklin's specimen was indeed a gynandromorph, it follows that the same unusual phenotype can rest on quite different genotypes, resulting from disturbances in the sex-chromosome : autosome ratio, a deficient **X**-chromosome as in Macklin's case and a deficient autosome as in this. This bird and also Miss Macklin's bird furnish evidence that in the fowl there are genetic factors which influence the size of the individual as a whole, and of local parts.

Alternative explanations of this case, though reasonable, involve so many assumptions that they are not so likely to be correct as is that suggested above. If fruitful polyspermy had occurred the peculiarities of this bird could be explained. One sperm (**wX**) would fuse with the egg-nucleus (**WX** or **WY**), while the other would remain alone in the egg-cytoplasm. Two nuclei, one **WwXX** or **WwXY** and the other **wX**, would thus be formed, and from these two cells the two sides of the body would be formed. If non-disjunction of the autosomes carrying the factor for white (**W**) and of the sex-chromosomes (**XY**) occurred during the maturation of the egg, and if thereafter two spermatozoa, each carrying an **X**-chromosome and an autosome with yellow (**w**), fertilised this egg, one sperm nucleus fusing with the egg nucleus and the other remaining alone in the egg-cytoplasm, then two nuclei would result, one **WWwXXY**, and the other **wX**. The first would give rise to tissues which were triploid (and therefore larger), male (since they would be **XXY**), and white (since white is dominant to yellow); the other daughter-cell would give rise to tissues which were haploid (and therefore smaller), female (since they contained only one **X**-chromosome), and yellow since no factor for white was present. Partial fertilisation cannot account for the production of the individual, but if the mother had been heterozygous for her epidermal pigment character, then the synchronous fertilisation of two egg-nuclei or egg-nucleus and nucleus of a polar-body, **WX** or **wX** and **wY** or **WY**, by two **wX** sperms might account for the condition. Cytological examination of the two testes revealed no difference in chromosome content.

SUMMARY.

A fowl with the left side of the body larger than the right is described. It is shown that, though the size difference is similar to that distinguishing male and female this bird was most probably not a gynandromorph.

The lateral asymmetry is explained on the assumption that an autosome carrying white had been eliminated during the early cleavage divisions of a male zygote heterozygous for the characters white and yellow epidermal pigmentation.

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EXPLANATION OF PLATE III.

Figs. 1 and 2. Illustrating the peculiar stance due to the different length of the bones of the two sides of the body.

Fig. 3. The bones. The two bones of a kind are placed together, left above right. Sternum and pelvis are curved toward their right sides. The ribs are placed in parallel series, the left ribs being to the right.

PLATE III
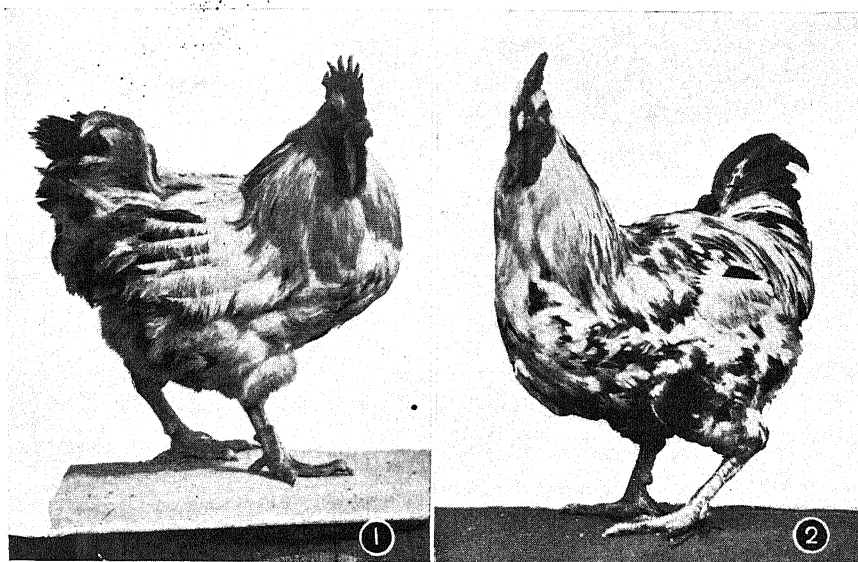
Figs. 1 and 2.
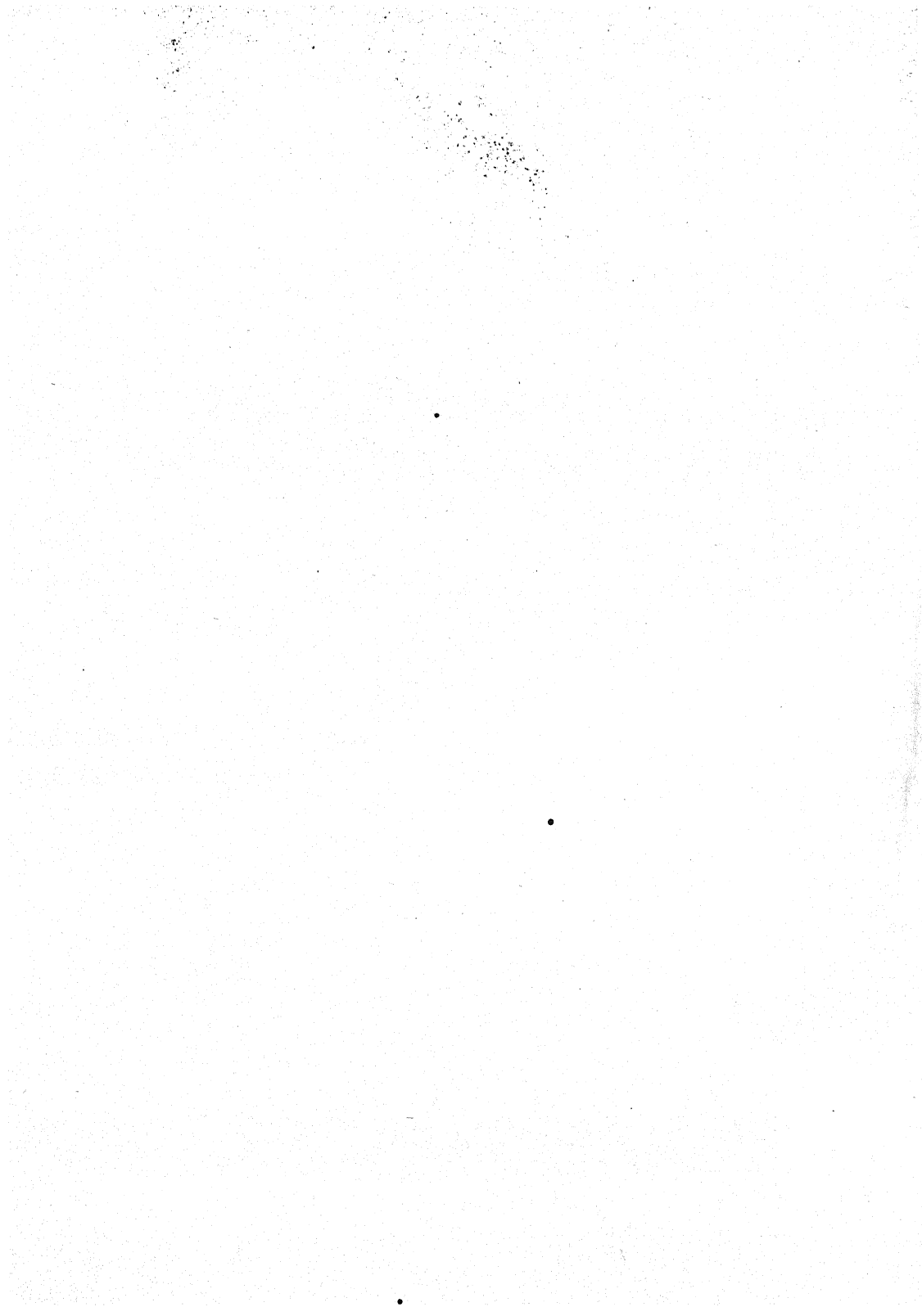
AMPUTATED, A RECESSIVE LETHAL IN CATTLE; WITH A DISCUSSION ON THE BEARING OF LETHAL FACTORS ON THE PRINCIPLES OF LIVE STOCK BREEDING.

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(With Three Text-figures.)

CONTENTS.

1. INTRODUCTION.

In a recent paper (Mohr and Wriedt, 1928) we described the mutant "hairless," a new recessive lethal in cattle. This gene is widely distributed within the Swedish breed of Holstein-Friesians (Svensk Laagland cattle). In the course of the investigation of this character, Mr Erik L. Larsson, adviser at the Hallands Läns Hushaallningsselskap, Falkenberg, Sweden, to whom we are indebted for the information concerning the occurrence of hairless calves, informed us that abnormal calves of quite another type also occurred within the same breed.

From the preliminary description there was some suspicion that these latter calves might be more or less similar to the "bulldog" calves in the Norwegian Telemark breed, studied by the authors (see Wriedt, 1925; Mohr, 1926). We therefore asked Mr Larsson, if possible, to send us a specimen, which he was kind enough to do in November 1927.

The individual received was entirely different from the above-mentioned bulldog calves, and exhibited malformations of such an extraordinary type, that we were at first almost inclined to doubt

whether we were dealing with a hereditary character. Nevertheless, on systematical inquiry the hereditary nature of the case was soon made perfectly clear. We are dealing with a single recessive lethal (or sub-lethal) gene which in the homozygous condition produces the very remarkable abnormalities described below.

Moreover, it soon turned out that quite a few among the most prominent sires within the Swedish breed of Holstein-Friesians had been heterozygous for this fatal gene, which accordingly now thoroughly infects the breed. In view of its great practical importance we shall in this paper give an account of the case, even though the anatomical investigation must as yet be of a purely preliminary nature.

Seen in connection with the above-mentioned parallel case, dis-covered practically at the same time, our data give a very striking picture of the spreading of lethal factors within a breed of domesticated animals. We are inclined to believe that observations like these must necessarily induce modifications in the breeding principles now prevailing, a point that we shall deal with at some length in the present paper.

We wish to express our sincere indebtedness to Mr E. L. Larsson both for the very valuable material provided by him, and also for his most generous and able assistance in the collection of pedigree data. We wish likewise to thank Mr Lars Slagsvold, Avdelingschef at the Veterinärinstitutet, Oslo, who kindly carried out the autopsy of the abnormal calf described below. To Mr Nils Wassberg, adviser at the Malmöhus Läns Hushaallningsselskap, we are also indebted for valuable information.

2. Description of the Amputated Calves.

According to consistent information from numerous breeders the abnormal calves are very uniform in appearance. We have had an opportunity of showing the pictures of the calf described below to quite a few herd owners, and all agreed that the abnormal calves which had occurred in their herds were strikingly similar to this individual. They also agree that the abnormal individuals are full-term, and that they are either stillborn or die immediately after birth.

We know of only one exception to this rule, an amputated calf born at the farm Boslid in Halland (see p. 200). This individual was fed by aid of a bottle, kept alive for two days and then slaughtered. It was described as having "short legs, without hoofs, and a short, chopped off head." Since this is the only case that differs somewhat from the others, especially as regards the degree of viability, we may probably consider

the individual now to be described as representative, though our description may perhaps be subject to minor modifications when more material is available.

This individual (Text-fig. 1), a full-term male calf, was received from Mr E. L. Larsson, November 23, 1927. His mother was the cow Siri, his father the bull Mac Ante 12499 (see p. 195).

Before it was sent to us, the calf had been injected with a formaline solution. On its arrival the weight was 25·5 kilo. The total length as measured from the top of the head to the point of the buttock was 72·5 cm. The calf had every sign of being born at full-term. The hairs were long and tight all over the body.

The colour is of the ordinary Holstein-Friesian pattern. The head and the neck are black with a large white star that is continued rostrally into a blaze. There is a black area on the left side of the abdomen and on the middle of the tail, and a larger black area along the middle of the saddle. With these exceptions the individual is white all over.

The general appearance of the individual is very odd, due to most extraordinary malformations of the head and of all four extremities.

The head. The eyes are protuberant with small eyelids. The ears are very short (right ear 5 cm., left 4 cm.); they are adjacent, and asymmetrical in position.

The face is entirely deformed (Text-fig. 2). Particularly striking is a sudden bend of the upper part of the face, commencing about 16 cm. anterior to the top of the head. From this point the profile turns downward almost at a right angle, thus giving the upper jaw a "parrot-bill"-like appearance. Nostrils are present; the left nostril is connected with the left angulus medialis oculi by a shallow furrow.

There is a total palatum fissum, processus palatinus maxillae and ossis palatini lacking on both sides. Dissection proved that the ossa intermaxillaria are loose and bent downward. There are large, rugged, not fully developed molars in the maxilla on both sides. The orbital wall proved to be open laterally. Cavum nasi is broad, with rudimentary conchae on both sides. The septum nasi is well developed. The deformed muzzle turns somewhat to the left.

The lower jaw is very rudimentary. On the left side the mandibula is only represented by an isolated roundish bone of the size of a fingertip, located in the foremost part of the lower jaw. On the right side the corresponding bone is of slender shape, irregular, of the size of a little finger. The ramus mandibulae is entirely lacking. Three fully developed, irregularly implanted teeth are found in the foremost part of the lower

Text-fig. 1. Abnormal calf, homozygous for the gene for *akroteriasis congenita*.

jaw, one on the left, two on the right side. No other teeth or rudiments of teeth are present in the lower jaw.

As a result of these radical malformations the cavity of the mouth is very short. From the very short upper jaw the limit of the oral orifice

Text-fig. 2. Head of abnormal calf.

runs as a straight line in caudal direction. Along this border line the mucous membrane of the cavum oris is everted to a considerable extent. Around the above-mentioned, irregularly implanted teeth the mucous

membrane covers some very irregular, fungus-like prominences (see Text-fig. 2).

In striking contrast to the cavum oris, the tongue is perfectly normal, both in size and in shape. Accordingly, the tongue which is much longer than the oral cavity projects from the oral orifice in a way that gives the entire face a fantastic appearance.

The extremities. All four extremities are lacking, the fore legs ending at the articulatio cubiti, the hind legs at the hock joint (Text-fig. 1)[1]. The ends of these vestigial extremities are bluntly rounded and covered with hairy skin like the rest of the individual.

Dissection proved that the scapula is fairly well developed, except that the spina scapulae is missing. The scapulae are connected with the trunk by the usual muscles. Also the humerus is relatively well developed. The cartilages of the cavitas glenoidalis scapulae and of the caput humeri have grown together and the joint is anchylosed, the scapula and the humerus forming a right angle.

The distal end of the humerus is fairly normal in shape. It is covered with cartilage, surrounded by a loose connective tissue in which there is an indication of bursa formation. The muscles originating from the scapula have tendinous ends which shade into this connective tissue around the distal end of the humerus. In the shoulder region the blood vessels and the nerve plexus were in the main normal.

The pelvis is normal and so is also the hip joint. The femur is fairly well developed. The muscles above the stifle joint are normal. The stifle joint is anchylosed, the tibia having grown together with the lateral condylus of the femur. Also the patella has fused with this condylus. Below the stifle joint were found a comparatively well-developed tibia and traces of a fibula. Distally the tibia ends in a somewhat thickened, roundish terminal part which is in direct contact with the skin. In the proximal part of the gaskin there were indications of a M. extensor digitorum communis with a tendon which was lost in the periosteum of the distal part of the femur. With this exception there were no muscles in the gaskin.

When the skull was opened it was found that the cranial cavity was enlarged. The same was true of the ventricles of the brain. The ventricles are filled with liquor to such an extent that the cerebral matter is reduced to a thin capsule with flattened gyri. There is in other words a pronounced hydrocephalus.

[1] According to later information concerning a particular herd in which 16 cases of amputated calves have occurred, a few of these had apparently normal hind legs.

The rest of the individual, including the viscera, were apparently normal. Macroscopical examination revealed nothing abnormal in the structure or size of the gonads, the thyroid gland, the hypophysis, or other glands with internal secretion. The material was not good enough for a microscopical examination of these organs.

On these findings, Mr Slagsvold gave the following diagnosis: Monster; Atrophia mandibulae and maxillae (perognati); Palatum fissum; Peromelia anterior (abrachi); Peromelia posterior (adachtyli); Hydrocephalus.

We have searched the literature for parallel cases without success. The above combination of malformations has apparently never been described. As a simple designation for this type of monster we propose the term *acroteriasis congenita*, suggested by Mr E. Smith, docent in Greek at the University. This term means: amputation of the protrusive parts.

That an individual with such numerous radical malformations should not be viable seems very natural, even though we have as yet not been able to point out the direct cause of death. This part of the investigation will have to be postponed until we have been able to secure sufficient and well-fixed material of the internal organs.

The case represents a very striking example of the "pleiotropic" action of a Mendelian gene, a property that is typical to all genes, only more or less marked in degree (see Mohr, 1926).

3. HEREDITARY TYPE OF THE MALFORMATION.

The data presented below are derived from the stud books of the farms involved, and were collected on the spot by Mr Larsson and Mr Wriedt. They demonstrate very clearly the hereditary nature of the malformation described. An important result of these inquiries is the fact that the gene in question can in every case be traced back to the very prominent sire Gallus M. 77[1], perhaps the most famous bull within the Swedish breed of Holstein-Friesians.

Gallus M. 77 was born in Ostfriesland (Germany) in 1890 (?). In Sweden he was first used for breeding purposes during the years 1892–1900 by Mr C. Maage, tenant at the farm Borreby in Skaane. In 1899 Mr Maage held a public auction, and the bull was bought by Mr August Kinck, the landowner at the farm Belteberga in Skaane. Both these farms sold breeding stock on a large scale, and sons of Gallus M. 77 were accordingly spread over the whole distributional area of the

[1] M. = Provincial Herd book of Malmöhus Län.

Swedish breed of Holstein-Friesians. Mr Kinck has informed us that he obtained four or five malformed calves of the above type by inbreeding within his herd. Mr Nils Wassberg, adviser at the Malmöhus Läns Hushaallningsselskap, states in a letter that 11 daughters of Gallus M. 77 were at Belteberga mated to four different sons of the same bull. Also other cases of inbreeding are known in this herd.

A son of Gallus, the bull Gallus Ödipus R. 7285[1], was sold to the farm Sannarp in Halland. No abnormal calves occurred among the offspring of this bull. But when his son, Gallus Haakon R. 7451, was mated to his own half-sisters from Gallus Ödipus 7285, there occurred in 28 calvings 22 normal and 6 abnormal calves (i)[2].
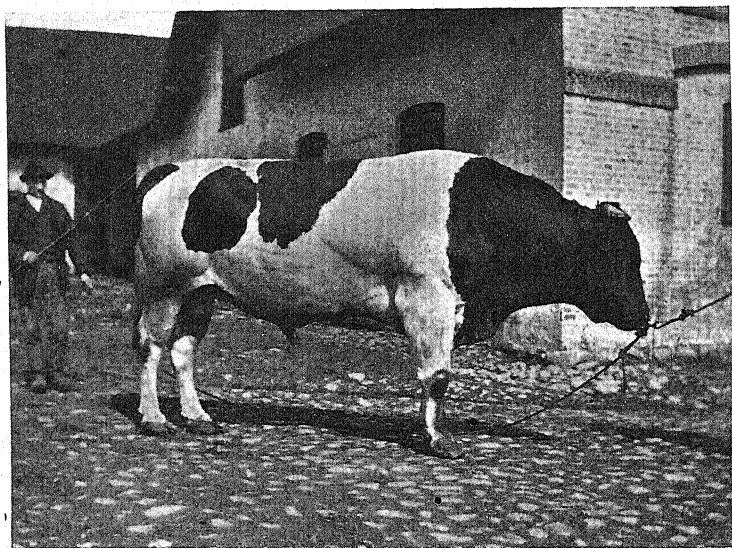
Text-fig. 3. The bull Gallus M. 77.

Another son of Gallus Ödipus 7285, the bull Gallus Galant R. 7362, did not carry the lethal gene. This is demonstrated by the fact that not a single abnormal calf occurred in 133 matings of this bull to cows, of which quite a few were his own half-sisters from Gallus Ödipus 7285.

A third son and a daughter's son of Gallus Ödipus 7285 have each given one abnormal calf when mated to daughters of the same bull.

At the farm Maanstorp in Halland the bull Ante Quidam R. 7630 has in 74 matings to his own daughters given 68 normal and 6 abnormal

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[1] R. = General Herd book of the Swedish Ministry of Agriculture.
[2] See collected data on p. 197.

calves (ii). This bull was also a prominent sire; he obtained both the first individual prize and the first breeding prize. From the pedigree (Table I) it is seen that Gallus M. 77 is the grandfather's father of Ante Quidam 7630.

TABLE I.

Pedigree of the bull Ante Quidam R. 7630.

Ante Quidam R. 7630	Belteberga Quidam R. 3246	Nobel Gallus	Gallus M. 77
			Natalia
		Qualitet	Nederland
			Quelle
	Annette	Pekema	
		Annette 2	President Perier
			Annette

A son of Ante Quidam 7630, the bull Mac Ante R. 12499, was mated to his own half-sisters from Ante Quidam 7630, and gave in 10 matings 9 normal and 1 abnormal calves (iii). With a daughter's daughter of Ante Quidam 7630 he gave in addition 2 abnormal calves, and in matings to the cow Ottelina R. 29597, 1 normal and 1 abnormal calf. As seen from the pedigree of her mother, also named Ottelina (Table II), this cow descends from Gallus M. 77 (fifth generation).

Finally, Mac Ante 12499 in matings to the cow Siri H.I. 2267[1] has given two abnormal calves. As seen from the pedigree (Table III), the bull Belteberga Quidam R. 3246, father of Ante Quidam 7630 (see pedigree, Table I), was the grandfather's father of this cow. In her pedigree we also find Gallus M. 77 on the maternal side (fourth generation). Mr Larsson learned through the prominent breeder, Mr Lars Trulsson, Lasseboda, Simlinge, that Belteberga Quidam has given amputated calves.

The same cow, Siri H.I. 2267, had previously also been mated to the bull Thomas R. 10746, and gave by him an abnormal calf of the same type. This bull cannot be seen to descend from Gallus M. 77. But an ancestress of his in the fifth generation backwards, the cow Herta M. 262, was born at the Borreby farm, where Gallus M. 77 served

[1] H.I. = Provincial Herd book of Halland Län.

at the time. In the herd book the bull Pontus is denoted as her father, but it is possible that Gallus M. 77 also may have covered her mother, the cow Hilda. Since this cow was imported from Ostfriesland, there is, however, also the possibility that she may have been related to Gallus M. 77 and may have carried the gene herself.

In three matings to daughters of the above-mentioned bull Thomas 10746, Mac Ante 12499 gave three normal calves (iv).

<p style="text-align:center">TABLE II.</p>

<p style="text-align:center">*Pedigree of the cow Ottelina, mother of the cow Ottelina R. 29597.*</p>

Ottelina	Belteberga Magnus	Prins Nikolaus		
		Margaret	Nobel Gallus	Gallus M. 77
				Natalia
			77 Molly	
	Ottelina	Max		
		Otilda	Sture f. Sturesholm	
			21 Otilda	

These data clearly indicate that we are dealing with a single autosomal recessive gene, which in the homozygous condition produces the peculiar malformations described. We have in all four sets of data derived from matings of heterozygous bulls (Gallus Haakon 7451, Ante Quidam 7630 and Mac Ante 12499) to daughters of heterozygous bulls. On the single

recessive factor basis we expect in such matings a 7 : 1 ratio of normal and abnormal calves. The actual result was as follows:

		Normal	Abnormal
(i)		22	6
(ii)		68	6
(iii)		9	1
(iv)		3	0
	Total	102	13

TABLE III.

Pedigree of the cow Siri H.I. 2267.

Siri H.I. 2267	Daniel Quidam	Dewan Quidam	Belteberga Quidam R. 3246	Nobel Gallus (ex **Gallus M. 77**)
				Qualitet
			Dora	
		Dveka	Staf	
			Dveka	
	Siri	Belteberga Gert	Quintus Gallus	**Gallus M. 77**
			Grete	
		Sivan	Adolf Bertram	
			Sara	

Thus, in 115 matings, 102 normal and 13 abnormal calves were obtained, a result which is in perfect accordance with expectation (101 : 14).

In addition to the above data we have also collected the following information on the occurrence of amputated calves:

TABLE IV.

Pedigree of the bull Ideal Herman R. 10310.

Ideal Herman R. 10310					
Ideal Aster	Ideal Peter	**Peter Jean** R. 5247			
		Ingrid			
	Astrid	Kasper	Harum	Zar Boris	
				Kustan	
			Kejserinnan	Petter	
				Karin	
		Asta	Belteberga Ome	Nobel Gallus (ex **Gallus** M. 77)	
				Opalia	
			Anastasia	Nobel Gallus (ex **Gallus** M. 77)	
				Krona	
Harmonie 49	Krüger	Albert II			
	Harmonie 37	**Prins Adolf** R. 3408	Cesar III		
			Weiser XX		
		Harmonie 17	Pr. Krüger		
			H. 11	Holly	
				H. 7	

Among the offspring of the bull Ideal Herman R. 10310, belonging to the Eldsberga-Öringe Bull Association, there occurred three abnormal calves. In his pedigree (Table IV) the bull Nobel Gallus, a son of Gallus M. 77 (see pedigree, Table I), occurs twice in the fifth generation. Attention should be called to the fact that we find in the same pedigree the bulls Peter Jean R. 5247 and Prins Adolf R. 3408, in the third generation. As shown by the authors (Mohr and Wriedt, 1928) these bulls were heterozygous for another lethal, the gene for hairlessness. There is accordingly the possibility that Ideal Herman 10310 may have carried both these lethals in heterozygous condition. So far, however, we only know of amputated calves occurring among his offspring. Out of the three cases that have come to our knowledge, one was derived from a mating to the cow Elina, the other from a mating to a daughter of this cow. The cow Elina has Gallus M. 77 among her ancestors in the fifth and sixth generations. The third abnormal individual occurred in a mating of Ideal Herman 10310 to one of his own daughters.

TABLE V.

Pedigree of the bull Bankett R. 11863.

Bankett R. 11863	Olaus Banbo	Banko Peter	**Peter Jean** R. 5247
			22 Basta
		Olgina	
	Nenette v.d. Meer		Belteberga Quidam R. 3246
			Qualitet
		Zwarte v.d. Meer	

Another bull, Bankett R. 11863, at the farm Karlsfält in Halland, who was a daughter's son of Ante Quidam 7630 (Pedigree, Table I), has in matings to daughters of the bull Ideal Herman 10310 (Pedigree, Table IV) given two amputated calves. The grandfather's father of Bankett 11863 was the bull Peter Jean 5247 (see Pedigree, Table V). It is therefore possible that Bankett 11863 may have carried both lethals. The same is true of the bulls Finn Fernando 8525 and Lord

Wellesley 10648 which both have Gallus M. 77 in their pedigree (fourth generation). Both these bulls were heterozygous for the gene for hairlessness (see Mohr and Wriedt, 1928).

According to information obtained from Mr E. L. Larsson, four amputated calves have also been born at the farm Lynga in Halland. Their father is the bull Lord Ante R. 11327, a son of Ante Quidam 7630. Mothers of these calves were the following cows: Betty (Farm No. 265), Vera (180), Gottlieb (23) and Mina (202). The maternal grandfather of Betty (265) was the bull Arne Kvick R. 7390, and this bull was also the father's father of Vera (180) and of Gottlieb (23). Arne Kvick 7390 is the son of a son of Gallus M. 77. The paternal grandfather's father of Mina (202) was the bull Belteberga Quidam 3246 mentioned above (p. 195). Out of the four last-mentioned cows, three came to Lynga from the herd of the Berte Quarn Stock Company, Slöinge, the herd in which the lethal factor for hairlessness was first discovered (see Mohr and Wriedt, 1928). Thus, here also there is the possibility that both these lethal genes may have been present in one and the same individual.

In all the above-mentioned 28 cases, except one, it is possible to trace the pedigree back to Gallus M. 77, both on the paternal and on the maternal side. The only exceptional case is the abnormal calf occurring among the offspring of the bull Thomas 10746. This case has been discussed above (p. 195).

Finally it should be added that six amputated calves have occurred among the offspring of the bull Solid R. 9717 at the farm Boslid in Halland. His paternal grandfather's father was the bull Belteberga Quidam 3246 (see Pedigree, Table I). One of these calves was exceptional in so far as it lived for a few days after birth (see p. 188). It has not been possible to secure pedigrees of the mothers of these abnormal calves, the records of this herd being incomplete.

After this had been written, Mr Larsson informs us that he has later learned that analogous amputated calves have also occurred in no less than four large and six smaller additional herds, not mentioned above, a fact that illustrates still more strikingly the very widespread distribution of this lethal gene[1].

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[1] While this paper was in the press one of us (Chr. W.), while on a visit to Skaane, happened to obtain information concerning four additional herds in which amputated calves have occurred (23 cases). In all of these cases it was possible to trace the pedigree back to Gallus M. 77 on both sides. In one of these herds a typical case of congenital hairlessness also occurred.

4. GENERAL CONSIDERATIONS AND DISCUSSION.

(a) *The influence of the great sires in live stock breeding.*

The development of the means of intercourse and the increasing economical importance of live stock breeding have during the last century led to an ever-increasing trade in breeding animals all over the world.

From the beginning of the 19th century the development of the different breeds of economical importance has to a marked extent been influenced by the practises of public shows. The first public show was, so far as we know, a show that, according to W. Gilbey (1904), was held in 1798 in Lewes, Sussex, England. The first public show in Denmark for all kinds of live stock was held in the year 1810; in Würtemberg the first public show was held in 1817.

About the same time the government in quite a few countries introduced the election of males to be used in breeding. Accordingly, the judges at the public shows elected those animals that had the phenotype which they considered appropriate. It soon turned out that only relatively few males gave offspring of the desired type. The result of this was that sons and grandsons of those males which proved to have the desired genotype were soon spread over the entire district of distribution of the breed in question.

Thus, not long after the formation of the Shorthorn breed, all cattle in England belonging to this type decended through one or more lines from the bull Favourite. When the Shorthorn breed of Cruickshank in the years 1876–89 came in fashion, the great majority of the Shorthorn animals in England soon showed descent from his leading sire, Champion of England 17526. To-day it is hardly possible anywhere in the world to find a Shorthorn animal that is not derived from this bull, through one line or another, and as a rule he occurs several times in the pedigree.

The influence of a single individual on the development of a breed is most strikingly illustrated in the Swedish red and white cattle (rödbrokig svensk fé). The origin of this breed is found in a single herd, the herd of the farm Stjernsund. This farm supplied bulls to practically every herd of this particular breed.

At Stjernsund the breed was built up on two sires, the Shorthorn bull Windsor, born at Alnarp in 1877, and the Ayshire bull Hero, born in 1880 in the herd of Mr Robert Paton, Trees Ayr. After Hero no new bull was brought to Stjernsund, and all later bulls at Stjernsund were

accordingly descendants of either Windsor or Hero. The direct male line of descent from Windsor is now extinct, and all red and white bulls now in existence are derived in direct male line of descent from Hero.

How frequently these bulls are represented in the pedigrees of red and white Swedish cattle is illustrated by an investigation carried out by Thesen (1914). He found that in the pedigree of a cow, born in 1912, Windsor was represented 30, Hero 18 times within six generations.

Analogous conditions prevail in all live stock breeds where stud books are kept. Thus, Mr Kjaer (1923), who examined the Register stud book of the Danish Jutland horse, found that among the 195 stallions and 1094 mares registered, no less than 189 stallions and 1006 mares descended in direct male line from the stallion "Oppenheim," born in England in 1859. The history of this stallion is rather extraordinary. He was in a consignment of Suffolk stallions, bought by the German horse dealer Oppenheimer, for delivery to the state of Mecklenburg. On arrival he was found to suffer from navicular disease, and was accordingly not accepted by the state. Mr Oppenheimer, who had also trading connections with Jutland, sent him to this Danish province, where he soon was very much used. In spite of the fact that the offspring of this outsider were not eligible for a premium, three sons of his turned out to be prominent sires, which through their offspring greatly influenced the development of the Jutland breed.

Among the above-mentioned stallions and mares descending in direct male line from "Oppenheim," no less than 187 stallions and 967 mares are derived in direct male line of descent from the stallion Munkedal, born in 1883. Of these again, 161 stallions and 841 mares descend in direct male line from the great sire Aldrup Munkedal, born in 1893, who may be regarded the actual founder of the modern Jutland horse.

In the two famous horse breeds in which the breeding has not been influenced by show selection, the situation is identical. In the English thoroughbred it is now all over the world impossible to find a horse that cannot be proved to descend from the stallion Stockwell, born in 1849. Similarly, the pedigree of every American trotter may be traced along different lines up to the stallion Hambletonian 10, born in 1849.

For further illustrations of the influence of a limited number of prominent individuals in live stock breeding we may refer to the large series of pedigree investigations issued by the Deutsche Gesellschaft für Züchtungskunde.

It is interesting to notice that the breeders have comparatively early been aware of this situation. Thus, Stonehenge (1867) writing on grey-hounds states that: "Lancashire has still some strains peculiar to herself, which have suffered no intermixture for many years, and the same may be said of the Yorkshire blood; but these are exceptions to the general rule, for nine-tenths of the greyhounds in these districts are now crossed with Scotch or Newmarket blood, through 'King Cob,' or 'Jason,' or some of their descendants. Indeed, it is now extremely rare to meet with any first-class breed of greyhounds which has not the name of one or other of these dogs in their pedigrees; and, as in former years it was thought necessary to trace every dog if possible up to 'Snowball,' so now, if it can be asserted that a favourite is descended from 'King Cob,' it is considered that a good claim to high breeding has been made out."

Genetically the consequences of the situation outlined above are as follows: Since the descendants of a very limited number of prominent sires are within a short time so mumerous that they may be said practi-cally to constitute the entire breed, recessive factors present in the breed will not be eliminated if matings are restricted to random sampling among those individuals that are homozygous or heterozygous for the corresponding dominant allelomorph. If for some reason or other it is desired to purify the breed for a certain recessive gene, this goal cannot be attained merely by excluding those individuals that are homozygous for the recessive factor.

Starting with a population consisting of 25 per cent. of homozygous dominants, 50 per cent. of heterozygotes and 25 per cent. of homozygous recessive individuals, we should, by excluding the recessives, expect a gradual decrease in the number of individuals of this genotype. The formula for calculating the number of recessives is according to Jennings (1916) as follows: $\frac{1}{(n+2)^2}$; n being the number of generations. Corre-spondingly the heterozygous individuals are expected to decrease in number according to the formula $\frac{2(n+1)}{(n+2)^2}$. If this calculation is carried out it is found that after ten generations the number of heterozygous individuals is still 15·3 per cent., provided all the homozygous recessives have been eliminated during this time.

If there now turns up a prominent sire among these heterozygous individuals the result will be that the entire breed may be imbued with the recessive gene, even though inbreeding is intentionally avoided.

After say four or five generations, descendants of the sire in question will inevitably be mated, and homozygous recessive individuals will appear.

As a striking illustration we may take the recessive chestnut colour in the Norwegian Gudbrandsdals breed of horses. At the beginning of this century it was, purely as a matter of taste, decided that the colour of this breed ought to be either black or brown. And at the time it was comparatively easy to reduce markedly the number of homozygous chestnut horses for the simple reason that the two leading black sires, Dalegudbrand 446 and Draupner 613, did not carry the recessive chestnut gene.

But the next great sire within the Gudbrandsdals breed, Brimin 825, proved to be heterozygous for this gene, and through him the chestnut gene was soon spread to such an extent that the resistance to the chestnut colour had to be abandoned, and chestnut is now put on equal terms with black or brown. A single great sire may thus check all efforts in standardising a particular breed feature.

The situation is even more serious when the recessive gene involved is disadvantageous from an economical point of view, in the sense that it reduces the efficiency of the individual for a particular purpose. In the English thoroughbred we are, through the investigations of Robertson (1913), acquainted with a recessive gene belonging to this class, a gene that in homozygous condition produces a pronounced tendency to blood-vessel breaking, particularly in the cavum nasi. Such haemorrhages are especially likely to occur when the animals are strained. The anomaly is not analogous to human haemophilia, there being no disturbance of the coagulation process.

The first homozygous bleeder known is the stallion Herod, born 1748. He suffered from haemorrhage in two different races. Herod was a prominent sire, and after some time the majority of English thorough-breds traced descent from this stallion.

The next famous stallion homozygous for this fatal gene was Hermit, born 1864. His father, Newminster, descended from Herod through different lines, and among his offspring were other bleeders in addition to Hermit. The mother of Hermit, Seclusion, was herself a bleeder, and this was also true of one of her daughters. In spite of the tendency to bleeding, Hermit was a first-rate racehorse. He won the Derby in 1867 only a week after he had had a very severe haemorrhage. His offspring won no less than 846 races, with an aggregate amount of £356,699. In a head-line in the *Live Stock Journal Annual*, 1924, Hermit is characterised as "A king among stallions."

The next famous bleeder was the stallion Gallinule, born 1884. He was a daughter's son of Hermit. He won three races when two years old, but later the bleeding prevented him from winning another race. As a sire Gallinule fully equalled his grandfather. His offspring won £307,854 in 628 races. Among his offspring was Pretty Polly, probably the most prominent racer of this century. She won in 22 races £37,297.

In addition to Hermit, Humorist, also a Derby winner, suffered from haemorrhage. He died in his box from bleeding, 17 days after his victory in the Derby of 1921. Through his father, Polymelus, Humorist descended from the well-known bleeder Toxophilite. In his pedigree we find Hermit represented twice on the maternal side.

Robertson's investigation includes 185 horses suffering from haemorrhage, and of these 17 died from it.

(b) The occurrence and spreading of recessive lethals in live stock.

Such considerations on the spreading of undesirable recessive genes in live stock apply also to recessive lethals. The only difference is that the elimination of the homozygous recessive individuals takes place automatically since the recessive involved is a lethal gene. The result is identical.

From the investigations of Muller and Altenburg (1919, 1921) and of Muller (1923) we know that lethal recessive mutations are of far more frequent occurrence than are the non-lethal factors that produce somatic changes, and we should consequently expect to find recessive lethals of frequent occurrence in live stock. This is undoubtedly the case. That relatively few are as yet known is partly due to the fact that only the sub-lethal genes are likely to be detected, and partly to the fact that breeders are inclined to suppress information concerning the occurrence of sub-lethal deformities and anomalies in their herds. Lack of qualifications among veterinarians for a correct interpretation of such cases undoubtedly also plays a certain part.

Of the recessive lethals known in live stock[1] only one is strictly lethal, viz., the lethal gene that Dunn (1923) was able to analyse in the fowl, thanks to its linkage to the recessive white gene in a strain of White Wyandottes. The rest are sub-lethal.

Very instructive is the sub-lethal deformity in lambs, recently described by Roberts (1926). The limbs, especially the fore limbs, are contracted and perfectly rigid, so that it is impossible to bend them.

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[1] The dominant genes with recessive lethal effect are disregarded in this paper.

The anomaly almost invariably results in stillbirths. The defect is due to a simple Mendelian recessive. Cases were discovered in flocks widely distributed through the country involving two mountain breeds—one Longwool, and one Down.

Most interesting is also the sub-lethal factor studied in horses by Yamane (1925, 1927). In the homozygous condition this gene produces a complete *atresia coli*. The large intestine is entirely cut off in the region of its pelvic flexure. The homozygous individuals, without exception, die within 2–4 days owing to non-functioning of the large intestine. The *atresia coli* is frequently combined with *glioma cerebri*. This fatal gene was introduced into Japan from Ohio, U.S.A., 40 years ago through a Percheron stallion "Superb," a prominent sire. Stallions and mares descended from him are now spread all over the province of Hokkaido. In 1922 no less than 161, or 26·2 per cent., of the 6614 sires belonging to the heavier breeds were related to him, a fact that illustrates the rapid distribution of his descendance. Yamane's investigation comprises 25 cases of the anomaly which occurred among the offspring of six stallions, all descendants of "Superb," in matings to related mares. That the gene in question may have a wider distribution is demonstrated by the fact, that Nusshag (1925) described three cases of *atresia coli* which, in addition to a probable fourth case, occurred among the offspring of an East-Friesian stallion in the stud of the Rittergut Vosshof, Altmark, Germany. According to Yamane, these cases are in every respect identical with the ones studied by him.

In cattle three cases of sub-lethal recessive factors have previously been described. The first of these was detected by the authors (Wriedt, 1925; Mohr, 1926), in the Norwegian Telemark breed. In the homozygous condition the gene produces a very marked *achondroplasia congenita*. These "bulldog" calves are born alive, but since they are unable to stand up they generally die within a few days from respiratory paralysis.

We have been able to trace this gene up to the bull Niklas 481, born in 1899. According to information obtained from Mr Neri Valen, he gave bulldog calves in matings to his own daughters. Niklas 481 could not have been the first carrier of the gene, since analogous calves also occurred when he was mated to other cows. Niklas 481 was considered a valuable sire; he obtained the first breeding prize and served up to 1911. His sons, however, were not highly esteemed by the judges at the public shows, and accordingly only eight have been registered. The distribution of this gene has therefore so far not been very great within

the Telemark breed. Only in two counties, where association bulls descending from Niklas 481 were used, has the gene spread to an extent that may be considered directly harmful from an economical point of view.

Malformed calves of seemingly analogous type have been described by Weinkopff (1927) in Holstein-Friesians, in Germany. From Sweden, Mr Gyldenskjold Wallén informs us that calves have occurred within his Ayrshire herd that look strikingly like the pictures of our bulldog calves, but it is impossible to tell whether we are dealing with the same gene in the last-mentioned cases.

In Holstein-Friesians Hadley and Warwick (1927) have discovered a recessive sub-lethal gene that in the homozygous condition produces a skin anomaly, *epitheliogenesis imperfecta neonatorum bovis*. Forty-three such defective calves have been recorded in 13 different herds in Wisconsin, U.S.A. The pedigree studies showed that all these calves traced to the same foundation stock that was imported from Holland about 1871. The authors also quote information from Mr G. M. van der Plank to the effect that similarly defective calves occur in Holland in herds carrying the same blood-lines.

The third and fourth analysed cases of sub-lethal recessive genes in cattle are represented by the mutants hairless (Mohr and Wriedt, 1928) and the one here described, both detected at the same time in the Swedish breed of Holstein-Friesians[1].

The breeding of Swedish Holstein-Friesians has been managed in an exemplary manner according to the breeding principles now prevailing. Type selection has been carried out according to very rigorous regulations, and the point-judging has been systematised in an admirable way. Inbreeding has been avoided, "blood-refreshening" being the general rule. It would be very difficult to find any breed in which the public supervision of the breeding has been equally well administrated.

When this is remembered, the cases investigated by us are very instructive. The entire breed is now thoroughly imbued with two different lethal genes, the genes for *hypotrichosis congenita* and for *akroteriasis congenita*. This result is a direct consequence of the fact that of the two leading sires within the breed, one, the bull Prins Adolf 3408, was heterozygous for the lethal gene for congenital hairlessness, while the other, the bull Gallus M. 77, was heterozygous for the recessive lethal described in the present paper. Both these bulls were imported

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[1] After this had been written still three other typical cases have come to our knowledge.

into Sweden, the former from Friesland (Holland), in 1902, the latter from Ostfriesland (Germany) in the early 'nineties.

In order to give an impression of the general importance of these bulls the following statements of Hansson and Nanneson (1918) may be quoted: "Among those bulls which have most strongly influenced the Swedish breed of Holstein-Friesians, Gallus takes the first place. He served during the years 1892–1900 at the farm Borreby, and from 1900 to 1903 at the farm Belteberga. His strong influence proved to be particularly favourable for the exterior (the phenotype) of the offspring and also for their milking capacity. At the public breeding sale in Malmö there were sold, up to 1915, 44 sons, 250 grandsons and 319 great-grandsons of Gallus. During the later sales 70 to 80 per cent. of the bulls present had the blood of Gallus in their veins." "Other sires of great importance have been the Gallus-son Björn (at Näsbyholm) and the grandson of Gallus, Belteberga Quidam belonging to the Simlinge Breeding Society, as well as Prins Adolf from Näsbyholm." It will be remembered that of the four bulls here denoted as the most prominent sires within the breed, the two first have been shown to carry the gene responsible for the amputated monsters, while the last one carried the gene for hairlessness.

A still more striking impression of the importance of the great sires Gallus M. 77 and Prins Adolf 3408 is obtained from a table published by Funkquist (1927) in the annual report of the breeding society of Swedish Holstein-Friesians. This table gives a survey of the 23 bull-lines contained in the Family Herd book of Swedish Holstein-Friesians, comprising 8795 registered bulls. This table starts with the Gallus-line comprising 2881 bulls. Then follows the Prins Adolf-line with 2065 bulls. Thus, out of the 8795 bulls registered, no less than 5946 bulls, or more than two-thirds, belong to the Gallus- and the Prins Adolf-lines, while the 21 other bull-lines together comprise 2849, or less than one-third of the total number of bulls.

As we have only considered the bull-lines here it is superfluous to emphasise that the importance of the Gallus- and Prins Adolf-lines within the breed is much greater than is indicated by the above data alone. The actual situation now is that if we trace the pedigree of any Holstein-Friesian individual in Sweden, there is an overwhelming probability of meeting either Gallus M. 77 or Prins Adolf 3408 in the ancestry and in very many cases we meet with both.

We have not as yet carried out special investigations to see *how* widespread the two lethals now are within the breed. That they are

very widespread is, however, already obvious from the data now at hand. The leading herds within the breed were first the Borreby herd to which Gallus M. 77 belonged for many years, and later the Näsbyholm herd, in which Prins Adolf 3408 served. To give an impression of the importance attributed to these herds we may quote the following remarks of Leufvén (1919):

"Through the newspapers it is announced that the Näsbyholm herd of Holstein-Friesians (laaglandsstam) is soon to be sold at a public sale. The fears that were expressed in this *Journal* at the decease of the old Baron von Blixen Finecke, namely that the precious herd might be scattered, with irreparable harm to the country and the breed, now seem to be fulfilled. But this must not take place; the values thus at stake are so great that our country cannot afford to sacrifice them! All that lies in our power must be done in order to prevent this, the most prominent herd of the breed, from being scattered, and the value represented by the entire, homogeneous herd from being annihilated!" "It is sufficient to recall the fate that befell the Borreby herd when brought to the hammer some 20 years ago." Both the above-mentioned herds were centres for the spreading of recessive lethal factors.

The widespread occurrence of the gene for hairlessness is illustrated by the fact that at the Skaar farm, near Gotenburg, three sires that served after each other were all heterozygous for this gene. These bulls were bought in the course of a series of years at the public breeding sales at Malmö, the two latter for the purpose of "blood refreshening" (see Mohr and Wriedt, 1928). Similarly, we have encountered three cases in which the gene for *akroteriasis* has been carried by bulls that were purchased for the purpose of "blood refreshening" to herds in which this gene was already present. In ordinary usage all these bulls were unrelated to the cows of the herds in question. In view of cases such as these, and in view of the rapidly increasing information from herds in which malformed calves of one type or the other occur, we are evidently justified in stating that the two genes are now very widespread within the breed. The consequences of the extensive use of great sires that were heterozygous for recessive lethal genes can hardly be more clearly illustrated.

(c) *The economical importance of recessive lethals in cattle.*

It is evident that the presence of lethal recessive genes of the type here described involves a marked depreciation in value in all meat-

producing animals. In such animals the offspring represent the only, or at any rate the main product.

In milk-producing animals and in the horse the conditions are somewhat different. In milk-producing cattle the calf does not represent the all-dominating product, and in the horse the brood-mare is a working animal as well as a breeder.

On a superficial view it might seem to be of relatively little importance that every eighth calf is lost in a herd of milk-producing cattle due to the presence of a lethal recessive. But the elimination of 20 calves in 3 years within a single herd—an actual case encountered in our investigation—cannot be regarded as unimportant from an economical point of view. Moreover, the possibility of more than one sub-lethal gene being present within the same herd cannot be disregarded. Though we have not as yet discovered such cases, we have demonstrated that both the gene for *hypotrichosis* and that for *akroteriasis* have probably come together in one and the same herd.

However, the loss of every eighth calf is of very great importance from a *breeding* point of view. In Denmark, one of the most prominent milk-producing countries, we may, according to Fredriksen (1924), estimate that up to 20 per cent. of a herd are sorted out each year. In order to replace these individuals in a herd of 100 cows it is necessary to rear 22 heifer-calves annually. It must also be taken into account that at least 10 per cent. of the heifers are dropped due to diseases or infertility before the first calving.

According to the Danish statistics for the year 1923 there were born 86 calves per 100 cows. Half of these calves are heifers. Of these some are apt to die from diarrhoea and other diseases shortly after birth. According to Fredriksen, ca. 38 viable heifers per 100 cows in one year may be regarded a fair output.

Provided that the rearing of heifer-calves from any cow in the herd could be recommended, this would represent an ample margin, and the loss of every eighth heifer-calf would be relatively unimportant from an economical point of view. However, those herds are very rare in which every cow is satisfactory from a breeding point of view. In most herds one must be content if half of the cows are prominent enough as regards milking capacity and butter fat percentage to be used as breeders. Under these conditions less than 20 heifer-calves are at hand for rearing, and this number is too small for the renewal of the herd. It should also be remembered that the general physical condition of the calf must be taken into account.

Thus in milk-cattle the occurrence of lethal factors is of much greater economical importance than indicated by the butchery value of the eliminated calves. Moreover, the psychological consequences cannot be disregarded. The possibility of breeding expensive animals giving rise to malformed monsters and stillbirths is in itself apt to cause a marked fall in the prices paid.

(d) Practical measures for prevention of the spreading of lethals.

The cases encountered in the Swedish Holstein-Friesians demonstrate very clearly how, by the now prevailing breeding methods, an entire breed may be imbued with recessive lethal factors. That such factors are of far more frequent occurrence in live stock than formerly believed is also perfectly clear. Thus, in the course of eight months, no less than six such mutant genes have come to our knowledge, five in cattle, one in sheep. Under these conditions the important practical question arises, how they are to be removed from the genotype of the domesticated animals.

It will be noticed that in this paper we have restricted our attention to the recessive sub-lethal genes. If dominant factors with recessive lethal action are concerned, the situation is comparatively simple. The elimination of the undesirable gene is here attained by excluding every heterozygous individual from breeding.

In rapidly propagating animals, and in animals where the value of the single individual is comparatively small—for instance in rabbits and in poultry—the elimination of recessive lethals may be reached by the ordinary procedure of inbreeding and selection (see Mohr, 1926).

The same method is, however, not efficient in the slowly propagating and more valuable domesticated animals. The economical risk is here too great. In view of the very large importance of the great sires pointed out above, it is necessary to take special steps in order to prevent the spreading of undesirable genes of the type discussed. How rigorous these measures should be depends to some extent upon the particular case.

When the spreading of such a gene has been detected within a breed of cattle, individuals that have already proved to be heterozygous should of course as a general rule be excluded from breeding. But it is also necessary for prophylactic reasons to secure knowledge as to the genotype of those males that are selected to serve as breeders before they are allowed to be used as bull-fathers. This is attained by requiring

that any such bull should beforehand have given at least 20 normal calves in matings to his own daughters.

Before a bull has given 20 calves with his own daughters he will have reached an age of about 7 years. It is accordingly of interest to know how many registered bulls have fathers that are 6 years old or more, since bulls that are 6 years old at the birth of their sons may have given 20 calves with their own daughters when their sons are ready for sale. To get an answer to this question we have examined the Herd book of Norwegian Telemark breed, vol. i, Bulls (1926). We have here found that of the 511 bulls registered during the years 1910–20, 228, or 45 per cent., had fathers that were 6 years old or older. These data are entirely unselected. The result is a simple consequence of the fact that registered bulls have generally been used till they are old.

Hence it is doubtless practicable to require that only bulls that have been tested in the above-mentioned way should be permitted to serve as breeders on a larger scale. It should also be remembered that there is in milk-cattle a rich supply of bull-calves, since only one bull is needed for each 50 cows. Moreover, the practical measure suggested has the great advantage that at the same time we obtain information as to the genotype of the bull in other respects—transmission of milking capacity, butter fat percentage, etc.

Where the conditions are such as in the Swedish breed of Holstein-Friesians we are of the opinion that the above procedure ought to be given a wide application. Where the spreading of a lethal is restricted to certain more limited districts, the measures are of course of primary interest within the districts concerned. But since the gene may very likely have a wider distribution within the breed than as yet realised, bulls that promise to make great sires, *i.e.* bulls from which many sons are reared for breeding purposes, should always be tested systematically by inbreeding to their own daughters.

A consequence of the above is that bulls which have been tested by matings to 20 of their own daughters should always be given a marked preference when it is a question of state subvention to association bulls, etc. Sons of such bulls should also be given a preference to sons of non-tested bulls. Breeding prizes should only be awarded to bulls that have been tested by matings to at least 20 of their own daughters[1].

The application of more rigorous practical measures must necessarily,

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[1] When it is a question of testing the bull for a particular known recessive lethal, this test may be shortened by mating him with cows that are known to be heterozygous. This point we hope to discuss in a future publication.

especially at the beginning, be cautiously carried out. Cases *may* be encountered in which a sire that is heterozygous for an undesirable gene may already have proved to be so prominent in other genotypic respects that it is inadvisable to rule him out as a breeder. In such cases his sons should be tested before they are permitted to serve as breeders. Cases of this sort will, however, doubtless be very exceptional.

In the *horse* it would be too difficult to test a stallion by a sufficient number of his own daughters. Stallions and mares reach maturity so late that the stallion in question will be too old when a sufficient number of test matings have been carried out. When lethals or other undesirable genes are detected within a breed, heterozygous stallions should not be allowed to serve as sires, and inbreeding of individuals that may carry the gene should be evaded as far as possible.

In *swine*, where productivity is high and where the meat value represents the only product, a boar should be required to have given 6 litters with his own daughters before offspring of his are reared for breeding purposes. By this procedure at least 30 test individuals are obtained for the estimation of the genotype as regards factors that markedly lower the viability.

In *sheep* the requirements should be analogous to those suggested for cattle. This means that the ram will be 3 years old before he-lambs of his should be selected for breeding purposes. This practice involves not inconsiderable difficulties. But in meat-producing animals the economical consequences of the recessive lethal genes are great enough to justify the prophylactic measures suggested. Also in swine and in sheep it should not be forgotten that we obtain by this procedure valuable information as to the genotype of the individual in other important respects.

Finally, a system of recording suspicious cases of stillbirths and monsters in live stock should be arranged in order that the leaders of the breeding and the veterinary worlds may become acquainted with any occurrence of lethals in the different breeds.

SUMMARY.

A new recessive lethal gene, occurring in the Swedish breed of Holstein-Friesian cattle (Svensk Laaglandsboskap), has been described. In the homozygous condition the gene produces a series of radical malformations, here embodied under the general term *akroteriasis congenita*, i.e. amputation of the prominent parts. The pronounced atrophia maxillae is accompanied by palatum fissum, the mandibule being re-

duced to mere vestiges. The fore legs are "amputated" to the elbow joint, the hind legs to the hock joint. The autopsy revealed a pronounced hydrocephalus.

These monsters are full-term and normal size. They die immediately after birth. Only in one case was such a calf kept alive for two days.

Matings of four different heterozygous bulls to daughters of heterozygous bulls gave in all 102 normal and 13 abnormal calves of the type described, a result that is in perfect accordance with the expectation for a single recessive factor (101 : 14).

It is demonstrated that the gene was introduced into Sweden in the early 'nineties through the bull Gallus M. 77, who was imported from Ostfriesland, Germany. This bull is the most prominent sire within the entire Swedish breed of Holstein-Friesians, and the gene is now very widespread.

The influence of the great sires in live stock breeding is discussed in a special section, where illustrative cases in horses, cattle and dogs are considered. The previously known cases of recessive lethal factors in live stock are reviewed, and the spreading of lethal genes within a breed discussed. In cattle seven such cases are known, of which six have been encountered by the authors. Three of the latter cases have not yet been published. Particular attention is here paid to the illuminating cases in Swedish Holstein-Friesians, viz. the present case and the lethal congenital hairlessness, discovered at the same time and recently described by the authors in this *Journal*.

The latter gene was introduced into Sweden from Friesland, Holland in 1902, through the bull Prins Adolf R. 3408, one of the great sires of the breed. In the Family Herd book comprising 23 bull-lines including 8795 registered bulls, the Gallus-line comprises 2881 bulls, the Prins Adolf-line 2065 bulls. The 21 other bull-lines together include only 2849 bulls, or less than one-third of the total number.

The breeding of Swedish Holstein-Friesians has been managed in an exemplary manner according to the breeding principles now prevailing, and the result has been that the breed is now thoroughly imbued with two very undesirable sub-lethal genes.

The economical importance of recessive lethals in cattle is discussed, and practical measures for the prevention of the spreading of lethals in live stock are suggested. It is emphasised that lethal genes causing stillbirths and malformations are of far more frequent occurrence in live stock than previously suspected.

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SPECIES HYBRIDS IN *DIGITALIS*.

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A PAPER dealing with *Digitalis* species hybrids recently published in the *Journal of Genetics* (Vol. XIX, 1928, p. 269) appears to call for a supplementary note. The cross discussed in this paper is that between *Digitalis purpurea* and *D. ambigua* Murr., the latter synonymous with *D. grandiflora* L.

It may be pointed out in the first place that work on this hybrid has been considerably more extensive than the literature cited in the paper under discussion would suggest. In particular, attention may be called to the omission of any mention of Focke's (1881) experiments with these two species and also with others, of the work of Wilson (1906) in which *Digitalis purpurea*, *D. ambigua* (*grandiflora*) and *D. lutea* were used, and also of that of the present writer (1912) who gave in this *Journal* a very detailed comparative account of the cross *D. purpurea* × *D. ambigua* and its reciprocal.

From the records of crosses between different species of *Digitalis* (*D. purpurea* × *D. ambigua*, *D. purpurea* × *D. lutea*, *D. lutea* × *D. obscura*, *D. ambigua* × *D. lanata*), it may be concluded that not only do the F_1 plants show characters roughly intermediate between the two parent species, but that the F_1 plants from reciprocal crosses are unlike, each hybrid following the seed parent more closely than the pollen parent. As I have shown (*Journ. of Genetics*, Vol. II, 1912, p. 71) in the case of the *D. purpurea* × *D. ambigua* crosses, this leaning towards the maternal parent is evident in a considerable number of vegetative characters as well as in those of the flower. Among such vegetative characters may be mentioned stature, form of the inflorescence, shape of leaf, thickness of leaf, type of leaf venation and leaf margin. Perhaps the most striking instance of this tendency of the hybrids to resemble more nearly the seed parent is shown in regard to the red flower pigment. The *D. purpurea* plant I used was one of a pure-breeding deep magenta-purple strain. The flowers of the F_1 plants from the cross *D. purpurea* × *D. ambigua* were salmon purple in colour, containing a considerable amount of red sap pigment throughout the corolla, while those of the F_1 plant of the reciprocal cross were cream (lighter in shade than *D. ambigua*) with

only a pale rose flush above and very small dark purple spots within (see coloured Plate IV, *Journ. of Genetics*, Vol. II, 1912, p. 88).

This behaviour of *Digitalis* species crosses receives no mention in the paper now criticised, since the reciprocal cross was not obtained. The fact that the chromosome numbers of the parent species and of the F_1 hybrid of the *D. purpurea* × *D. ambigua* cross are now shown to be similar and that no segregation is apparent in the F_2 generation suggests that some form of cytoplasmic inheritance may be concerned. The increase in chromosome numbers in the F_2 plants is an observation of considerable interest, but does not appear to offer any help towards explaining the phenomenon.

It is also of interest to find that a cross *D. purpurea* (white var. no spots) × *D. ambigua* gave an F_1, all the plants of which bore flowers having a pink tinge. A similar result was obtained in a *D. purpurea* (white no spots) × *D. lutea* cross by Wilson, who drew the conclusion that colour may be latent in a white foxglove; but a more probable inference would be, perhaps, that *D. lutea* (or *D. ambigua*) carries a colour factor or some component of such a factor.

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BATESON'S EXPERIMENTS ON BOLTING IN SUGAR BEET AND MANGOLDS.

By Sir A. D. HALL, K.C.B.

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For some years the late Director of the John Innes Horticultural Institution, William Bateson, had been carrying out experiments on the inheritance of the "bolting" habit in sugar beet and other biennial plants. These experiments were in the first instance directed towards demonstrating the possibility of eliminating the bolting habit from the strains of seed to be grown commercially. At that stage it was not intended to study systematically the inheritance of the bolting habit but merely to ascertain if a genetic factor enters into it. The data are derived from Bateson's notebooks, but he left no record of the conclusions he had drawn from them. The object of the experiments is disclosed in Bateson's address to the Agricultural Section of the British Association in 1911: "I refer to the bolting or running to seed of crops grown as biennials, especially root crops. It has hitherto been universally supposed that the loss due to this cause, amounting in Sugar Beet as it frequently does to 5 or even more per cent., is not preventable. This may prove to be the truth, but I think it is not impossible that the bolters can be wholly, or almost wholly, eliminated by the application of proper breeding methods. In this particular example I know that season and conditions of cultivation count for a good deal in promoting or checking the tendency to run to seed, nevertheless one can scarcely witness the sharp distinction between the annual and biennial forms without suspecting that genetic composition is largely responsible."

The sugar beet, mangold and garden beet are all presumed to be derived from the wild *Beta maritima*, and, generally speaking, the strains in cultivation possess a biennial habit. In the first year of sowing they develop a leaf rosette and form the swollen root for which they are cultivated; in the second year the stem shoots up to carry flowers and seed. A certain proportion of plants, however, always throw up stems in the first year; these are known as "bolters" and commercially considered are valueless, so that their prevalence may seriously diminish the effective yield of the crop.

Climatic conditions have a marked effect upon the degree of bolting;

in certain seasons it is widespread. Early sowing followed by checks to growth promote bolting; if a late sowing is followed by unbroken growing conditions there are few or no bolters seen.

The procedure adopted was as follows. From a commercial stock seeds were sown under glass in December or January and the seedlings were planted out about the middle of April. At approximately the later date a control of the stock seed was sown in the open. Under these conditions the majority of the early seedlings planted out would bolt. A few of the non-bolters would then be stored out of reach of frost and in the following spring planted together in an insect-proof house or an isolated part of the garden under a muslin-covered cage. The members of this species are almost wholly self-sterile, but in a group of this kind, with a little shaking, will fertilise one another freely. The possibility of access of wind-borne pollen is not perhaps absolutely excluded, but with the absence of flowering plants of the same species in the district becomes exceedingly remote.

Seed derived thus, $A\,1$, $B\,1$, etc., was then subjected to the same treatment, partly sown under glass and partly in the open. Seed was again saved from the non-bolters among the plants which had been sown under glass, and the same selection was continued for further generations. In the following tables the varieties are distinguished by capital letters, families within the varieties by a repetition of the capital letters, and the successive generations of a family by figures after the capital letters.

Effect of Early Sowing.

In Table I are collected together the results obtained by sowing the unselected commercial seed of each of the varieties and strains dealt with under glass about the beginning of the year and then planting out the seedlings in April. Seed was also sown in the open about the same time.

The early sowing induced bolting in 5136 plants out of 7563, about 70 per cent., whereas the same seed sown in the open later only gave 91 bolters in 5417 plants, or less than 2 per cent. Sowing in December gives rise to a higher proportion of bolters than sowing in January or February, a significant fact. It is not necessary here to enquire further into the comparative bolting tendencies of the different varieties, the results simply demonstrate that the treatment (early sowing + planting out) is effective in revealing a potential tendency to bolting in all the strains, even though they show few bolters when grown normally.

TABLE I.

Effect of sowing early under glass and planting out commercial seed.

Variety	Year	Sown under glass		Sown in open	
		Date	No. of bolters	Date	No. of bolters
Mangold A	1916	11. xii. 15	8 in 19	—	—
„ A	1916	2. ii. 16	42 „ 317	25. iv. 16	2 in 390
„ B	1916	11. xii. 15	8 „ 28	—	—
„ B	1916	2. ii. 16	41 „ 291	—	—
Sugar beet C	1920	15. xii. 19	860 „ 1150	22. iv. 20	25 in 533
„ CC	1921	29. xii. 20	533 „ 603	13. iv. 21	10 „ 1000
„ CCC	1927	19. i. 27	148 „ 177	12. iv. 27	0 „ 130
Garden beet H	1924	20. xii. 23	509 „ 510	17. iv. 24	14 „ 466
„ H	1924	—	—	17. v. 24	0 „ 214
„ K	1924	20. xii. 23	428 „ 490	17. iv. 24	0 „ 582
„ K	1924	—	—	17. iv. 24	0 „ 131
Sugar beet D	1926	19. i. 26	233 „ 248	13. iv. 26	33 „ 196
„ D	1927	19. i. 27	156 „ 177	12. iv. 27	2 „ 226
„ E	1920	18. xii. 19	1162 „ 1563	22. iv. 20	4 „ 613
„ E	1921	29. xii. 20	541 „ 603	—	—
„ F	1922	20. ii. 22	174 „ 509	—	0 „ 312
„ G	1922	20. ii. 22	145 „ 435	20. iv. 22	1 „ 312
„ H	1922	20. ii. 22	148 „ 443	20. iv. 22	0 „ 312
		Totals	5136 in 7563		91 in 5417
		Percentages	67·9 %		1·7 %

INHERITANCE OF THE NON-BOLTING HABIT.

Table II summarises the results of the selection of seed from parent plants which did not bolt when subjected to the forcing treatment of sowing early under glass and subsequent planting out.

A is a family of Prizewinner Globe mangold which began in 1916 with 8 plants that did not bolt out of 19 plants sown under glass in the preceding December. The bolting tendency present in the seed is also seen by the occurrence of 2 bolters in 390 plants sown in the open at the end of April. None of the seed, saved from the non-bolters fertilised among themselves, bolted when sown in the open, nor when started under glass in mid-January 1918 (*A* 1). The second generation, *A* 2, saved from the *A* 1 non-bolters (*i.e.* no further selection because there were no bolters in *A* 1), again gave no bolters when sown in the open or under glass in mid-February. The next generation, *A* 3, did give 10 bolters in 136 plants sown under glass on 20 December. This was a severer test than had been applied to the *A* 1 and *A* 2 generations, which were only sown in January and February. A fourth generation, *A* 4, raised from the *A* 3 non-bolters, gave no bolters as a result of a sowing on 12 January. Further evidence that the original 1916 selection

had not completely removed the bolting strains is seen from later sowings of the *A* 2 seed. From seed sown on 5 January 1923, when 3 years old, 3 plants bolted in 414, though of the same seed none had bolted in 1920 when sown on 19 February. In 1927, when 7 years old, there were no bolters among 30 plants sown on 19 January, but the number was not big enough to make sure of including a bolter from the small proportion possibly present.

TABLE II.

Mangold, Sutton's Prizewinner Globe.

		Sown under glass			Sown in open		
Year	Family	Date of sowing	Date of transplanting	No. of bolters	Date of sowing	No. of bolters	
1916	*A*	11. xii. 15	27. iv. 16	8 in 19	27. iv. 16	2 in 390	Commercial seed
1918	*A* 1	14. i. 18	30. iv. 18	0 ,, 203	1. v. 18	0 ,, 193	Seed from non-bolters in *A*
1920	*A* 2	19. ii. 20	6. iv. 20	0 ,, 215	22. iv. 20	0 ,, 65	,, ,, *A* 1
1922	*A* 3	20. xii. 21	20. iv. 22	10 ,, 136	—	—	,, ,, *A* 2
1924	*A* 4	12. i. 24	24. iv. 24	0 ,, 213	—	—	,, ,, *A* 3
1923	*A* 2	5. i. 23	11. iv. 23	3 ,, 414	9. iv. 23	0 in 280	Seed 3 years old
1927	*A* 2	19. i. 27	11. iv. 27	0 ,, 30	12. iv. 27	0 ,, 51	,, 7 years old

Mangold, Sutton's Golden Tankard.

Year	Family	Date of sowing	Date of transplanting	No. of bolters	Date of sowing	No. of bolters	
1916	*B*	11. xii. 15	27. iv. 16	8 in 25	—	—	Commercial seed
1918	*B* 1	14. i. 18	30. iv. 18	0 ,, 182	1. v. 18	0 in 128	Seed from non-bolters in *B*
1920	*B* 2	28. i. 20	14. iv. 20	0 ,, 334	—	—	,, ,, *B* 1
1916	*BB*	2. ii. 16	27. iv. 16	41 ,, 291	15. vi. 16*	0 in 240	Commercial seed
1918	*BB* 1	14. i. 18	30. iv. 18	0 ,, 203	1. v. 18	0 ,, 272	Seed from non-bolters in *BB*
1920	*BB* 2	25. i. 20	14. iv. 20	0 ,, 276	22. iv. 20	0 ,, 276	,, ,, *BB* 1
1921	*BB* 2	20. xii. 20	19. iv. 21	7 ,, 737	12. iv. 21	0 in 750	Year old seed
1923	*BB* 3	5. i. 23	11. iv. 23	0 ,, 168	9. iv. 23	0 ,, 266	Seed from non-bolters in *BB* 2
1925	*BB* 4	12. i. 25	16. iv. 25	0 ,, 346	16. iv. 25	0 ,, 209	,, ,, *BB* 3
1927	*BB* 5	19. i. 27	11. iv. 27	0 ,, 45	12. iv. 27	0 ,, 108	,, ,, *BB* 4
1927	*BBB*†	19. i. 27	11. iv. 27	0 ,, 173	12. iv. 27.	0 ,, 260	,, ,, —

Sugar Beet, Vilmorin's B.

Year	Family	Date of sowing	Date of transplanting	No. of bolters	Date of sowing	No. of bolters	
1920	*C*	18. xii. 19	14. iv. 20	860 in 1150	22. iv. 20	25 in 533	—
1922	*C* 1	20. xii. 21	20. iv. 22	442 ,, 702	20. iv. 22	0 ,, 420	Seed from non-bolters in *C*
1923	*C* 1	5. i. 23	11. iv. 23	227 ,, 530	9. iv. 23	0 ,, 291	Repeat—year old seed
1924	*C* 2	20. xii. 23	17. iv. 24	310 ,, 434	17. iv. 24	0 ,, 151	Seed from non-bolters in *C* 1
1926	*C* 3	19. i. 26	12. iv. 26	0 ,, 250	13. iv. 26	0 ,, 206	,, ,, *C* 2
1927	*C* 3	19. i. 27	11. iv. 27	0 ,, 176	12. iv. 27	0 ,, 72	Repeat—year old seed

Garden Beet, Cheltenham Green Top.

Year	Family	Date of sowing	Date of transplanting	No. of bolters	Date of sowing	No. of bolters	
1924	*H*	20. xii. 23	22. iv. 24	428 in 490	17. iv. 24	0 in 582	Commercial seed
1926	*H* 1	19. i. 26	13. iv. 26	0 ,, 10	13. iv. 26	0 ,, 6	Seed from non-bolters in *H*

* Date of transplantation, was previously sown under glass 28. iv. 16.

† *BBB* was derived from a cross between the non-bolters of *B* 1 and *BB* 1, the two intermediate generations of which, growing in 1920 and 1922, were not subjected to test.

B is a family of Golden Tankard mangold. Sown under glass in December 1915, 25 plants yielded 8 non-bolters, seed from which did not bolt when sown in January 1918, *B* 1; nor again in the next generation, *BB* 2, sown on 25 January 1920. But this seed was not freed from bolters when tried out more severely. Sown on 20 December 1920 (being

a year old) it yielded 7 bolters from 737 plants. After this further selection, three generations, *BB* 3, *BB* 4, and *BB* 5, gave no bolters from January sowings, nor any naturally in the open.

Sugar beet was less readily freed from bolters. A strain which at the outset gave 70 per cent. of bolters under forcing gave 63 per cent. in the next generation and 71 per cent. in the second, though in each case the seed had been saved from non-bolters in the previous generation. In the third generation no bolters were obtained among 250 and 176 plants respectively in trials made in successive years. A single trial was made with garden beet (Cheltenham Green Top). Commerical seed gave bolters to the extent of 87 per cent. under forcing treatment, none in the open. The first generation from non-bolters gave no bolters under forcing, though the result is of less significance since only 10 plants survived.

From these trials it would appear to be comparatively easy to extract from the mixed population present in ordinary seed a selected group which breeds true to the non-bolting tendency, even when the seedlings are submitted to the drastic forcing test. The first selected generation of mangolds (Golden Tankard and Prizewinner Globe) gave no bolters, but it was not until the third generation of the sugar beet that the bolters were eliminated. The non-bolting character is not absolute. A more drastic forcing, by sowing in December, reveals some bolting tendency that had not been weeded out by breeding from non-bolters from January sowings, as shown by the recurrence of a few bolters in the second generation of Golden Tankard and the third generation of Prizewinner Globe. The sugar beet results are difficult to understand in that the selection seemed to effect no reduction in the tendency to bolt in the first two generations, *i.e.* 70 per cent. commercial seed, 63 per cent. first generation, 71 per cent. second generation, yet in the third generation the bolters appear to have been completely removed.

After the first selection none of the seedlings bolted under normal conditions of sowing in the open.

EFFECT OF AGE OF SEED AND OTHER FACTORS.

It has been suggested that there is an increased tendency to bolt with the age of the seed. Table III collects a few trials which bear upon this point.

The only evidence on the side of age increasing the tendency to bolt is afforded by *BB* 2 which gave no bolters in 1920, but which, when a year old, gave 7 bolters in 737 plants. This, however, was a December

sowing, whereas the 1920 crop was only sown in January, and such extra forcing was probably enough to reveal the few potential bolters. On the other hand, A 2 seed which gave no bolters in 1920 gave none also after keeping until 1927, and B 3 seed when five years old equally gave no bolters. Commercial sugar beet C stored for 7 years gave exactly the same percentage of bolters under glass, and actually fewer when grown in the open, compared with the proportion given 7 years previously, though the numbers in the latter case are not large enough to be significant.

TABLE III.

Effect of age of seed.

| Family | | Year | No. of bolters | |
			Sown under glass	Sown in open
Mangold	A 2	1920	0 in 265	0 in 65
,,	A 2	1927	0 ,, 30	0 ,, 51
,,	BB 2	1920	0 ,, 276	Failed
,,	BB 2	1921	7 ,, 737	0 in 750
,,	B 2	1920	0 ,, 334	—
,,	B 3*	1927	0 ,, 173	0 in 260
Sugar beet	C	1920	860 ,, 1150	25 ,, 533
,,	C	1927	86 ,, 117	0 ,, 94
,,	C 1	1922	442 ,, 702	0 ,, 420
,,	C 1	1923	227 ,, 530	0 ,, 291

* Not B 2 seed but seed harvested from B 2 non-bolters and 5 years old when sown.

INHERITANCE OF BOLTING.

Table IV summarises the instances in which seed was saved from bolters and the bolting family was carried on for one or two generations, breeding only from bolters.

Seed from Globe mangolds that bolted under the forcing treatment (a 1) bolted wholly when sown in December, a severer test than had been applied in the previous generation, but in the open did not bolt to a significant degree more freely than the original commercial seed. The selection (one-eighth of the total number of plants) had not been rigorous. On the other hand, seed (a 3) derived from the few bolters in the generation (A 2) that had been selected twice for non-bolting under glass, when again sown under glass gave 2 bolters in 8 plants, and gave 9 bolters in 131 plants in the open. This result is of some significance, since the parent seed in the two previous generations had given no bolters when sown in the open.

Seed of Tankard mangolds selected from bolters in the open (*aa* 1) all bolted under a December sowing, and bolted to the extent of 11 plants in 140 in the open, significantly greater than the bolting in the open of the non-selected parent stock (4 in 460).

TABLE IV.

Inheritance of Bolting.

Year	Family	Seed sown under glass			Seed sown in open		Source of seed
		Date of sowing	Date of transplanting	No. of bolters	Date of sowing	No. of bolters	
1916	Globe mangold *A*	2. ii. 16	27. iv. 16	42 in 317	28. iv. 16	2 in 390	Commercial seed
1917	,, *a* 1	4. xii. 16	25. iv. 17	130 (all)	25. iv. 17	3 ,, 190	Seed from bolters sown under glass
1924	,, *a* 3	12. i. 24	24. iv. 24	2 in 8	17. iv. 24	0 ,, 15 / 9 ,, 116	Seed derived from bolters in *A* 2, 1923
1923	Tankard *AA*	—	—	—	9. iv. 23	4 ,, 460	Commercial seed
1924	,, *aa* 1	12. i. 24	24. iv. 24	266 (all)	17. iv. 24	11 ,, 140	Seed from bolters in open
1922	Sugar beet *C* 1	20. xii. 21	20. iv. 22	442 in 702	20. iv. 22	0 ,, 420	Once selected from non-bolters under glass
1923	,, *c* 2	5. i. 23	11. iv. 23	25 ,, 43	9. iv. 23	0 ,, 19	Seed from bolters in above
1923	,, *C* 1	5. i. 23	11. iv. 23	227 ,, 530	9. iv. 23	0 ,, 291	Repeat, seed one year old
1924	,, *c* 2	20. xii. 23	17. iv. 24	117 ,, 124	17. iv. 24	0 ,, 30	Seed from bolters in above
1924	,, *c* 2'	20. xii. 23	17. iv. 24	45 (all)	17. iv. 24	0 ,, 23	Repeat
1922	Sugar beet (Red Top *F*)	—	—	—	20. iv. 22	2 ,, 312	Commerical seed
1923	Sugar beet (Red Top *f* 1)	6. i. 23	9. iv. 23	2 (all)	—	—	Seed from bolters in open
1924	Sugar beet (Red Top *f* 2)	20. xii. 23	17. iv. 24	356 (all)	17. iv. 24	104 in 251	Seed from above
1924	Garden beet *H*	20. xii. 23	22. iv. 24	509 in 510	17. iv. 24	14 ,, 466	Commercial seed
1925	,, *h* 1	20. i. 25	16. iv. 25	49 ,, 111	26. iv. 25	2 ,, 63	Seed from bolters under glass

With sugar beet *C* the stock had been once selected from non-bolters under forcing treatment but still gave more than half bolters when forced. Seed saved from one of these bolters still gave 18 non-bolters in 43 plants, a ratio almost identical with that of the parent stock. There were no bolters when sown in the open. The trial was repeated with a year old stock of the same seed, and this time there were only 7 non-bolters out of 124 plants. This, however, was a December sowing. In the open as before none bolted. With Red Top sugar beet seed was saved from one bolter out of 312 plants sown in the open. Only 2 seeds germinated under glass, both of which gave bolters. Seed saved from these wholly bolted when sown under glass and bolted to the extent of 40 per cent. when sown in the open. This is a case of pure line breeding from a single plant which bolted after being sown in the open, and the tendency to bolt in the open has been markedly increased.

Garden beet sown under glass bolted to the extent of 509 plants out of 510. Seed saved from these, which practically means unselected

seed, gave under glass only 49 bolters in 111 plants, less than the parent
unselected stock, but this is explained by the fact that the later was
a January, the earlier a December sowing. In the open it gave practi-
cally the same proportion of bolters as the parent stock.

Discussion.

From these experiments it is clear that both environment and
heredity play a part in inducing bolting. The stocks of seed which
show less than 2 per cent. of bolters when sown in April in the open
ground, show 68 per cent. of bolters when sown under glass and planted
out. Sowing in December induces more bolting than sowing in January
or February. Patent as is the effect of early sowing to induce bolting
it is yet possible by breeding a few generations from the plants which
do not bolt under the forcing treatment to obtain a strain that will not
bolt at all even though sown under glass. It is possible to find parallels
to this condition of a characteristic only being revealed under special
conditions of environment, *e.g.* Morgan has described an abnormal ab-
dominal condition in *Drosophila* which only appears when the food
supply is kept moist, and then behaves as a simple recessive character.
In this instance the data are not sufficient to allow of analysis of the
mode of inheritance of the bolting habit. Bolting cannot be regarded
as a simple character alternative to non-bolting because it only re-
presents a final stage of development through which all the plants must
pass. The distinction between annual and biennial is one of degree
rather than of kind. We have no knowledge of what determines the
plant at some stage to pass to reproductive development instead of
continuing its vegetative development. We may hypothesise the pro-
duction of a "hormone," or the attainment of a certain internal pressure
of some product of growth. Other things being equal the time required
for the plant to work up to this stimulus will be conditioned by the
environment. On the other hand, the degree of stimulus required to
initiate bolting, or the rate at which the stimulus is generated, is different
in different individuals, and this character is inherited. The results are
most readily interpreted by supposing the ordinary stock of seed to consist
of a mixed population of individuals possessed of different responses to
the stimulus of environment. A certain small proportion is impelled
to bolt under normal conditions; with earlier sowing a larger proportion
responds; a January sowing under glass followed by planting out excites
a still greater number; and only a small proportion can resist the drastic
treatment of a December sowing. Those that do resist give rise in

succeeding generations to seed that equally resists the weak stimulus of sowing in the open ground and the drastic stimulus of sowing under glass and planting out. The results obtained with commercial seed of Prizewinner Globe mangold in 1916 can be thus rearranged:

    0·5 % will bolt after sowing on 25 Apr.
    12·5 %   „   „   2 Feb. but not after sowing on 25 Apr.
    30·0 %   „   „   11 Dec.   „   „   2 Feb.
    57   % will not bolt when sown on 11 Dec.

It is not surprising that the ordinary stocks of seed should be so mixed as regards the bolting habit. Only a limited degree of selection has been practised, *i.e.* the rejection of plants that bolt in the open under normal conditions. Beet and mangold plants again are practically self-sterile, hence the seed originates almost wholly from cross-fertilised plants, and there is no chance of establishing lines pure for a character like bolting which is not being sought for by the savers of seed. If, however, attention is directed to this point it becomes possible to sort out of the mixed population a number of races, resistant to successive degrees of stimulus—at the one end "biennials" in that none of the individuals bolt even when forced, at the other end "annuals" in that all the individuals bolt when grown under normal conditions in the open. Confirmation of the mixed population conception is obtained from a consideration of the few trials in which seed was saved from bolters. Seed of mangolds saved from bolters wholly bolted when sown under glass in December, *i.e.* it was immediately possible to extract a race true to bolting under these drastic conditions. But the seed from bolters sown in December did not bolt any more freely in the open than the original stock, because this selection let through so many individuals bolting when sown early, but not bolting in the open, that it was in effect no selection at all. On the other hand, seed from bolters sown in the open did give a significantly greater number of bolters. That this seed did not yield only bolters shows how impure the cross-fertilised stocks were. This is again shown by the fact that the seed (a 3) selected from a few bolters after two generations that had given no bolters either under glass or in the open, gave 7 per cent. of bolters in the open, more than the original stock of seed. On the other hand, sugar beet C_1 never gave bolters in the open, and none were extracted from this stock by selection.

There is nothing to show that high resistance is dominant or recessive to low resistance; the evidence is rather that bolting is governed by several factors. Dudok van Heel (*Genetica*, 1927, IX, 217) states that the hereditary factors for bolting are recessive. According to his experiments

certain strains of sugar beet isolated by inbreeding show a tendency to segregate into families with a constant percentage of bolters. On crossing a high bolting family with a low bolting family the $F1$ generation gives a small bolting ratio. But it is difficult to accept the hypothesis of a pure line with a defined proportion of bolters. If it is a pure line it should either bolt wholly or not at all for a given environment. Conceivably a pure line might be so nicely attuned to the environment that some of a group would be pushed into bolting by the unavoidable variations in temperature, soil, water supply, etc. which must prevail, but the proportion so bolting would never be constant from season to season. As van Heel does not give the actual numbers it is impossible to discuss his results, as to the validity either of his pure lines, or the effect of crossing them.

The conception of a mixed population is consistent with certain results observed in practice. Some growers of sugar beet seed sow their stocks a month earlier than is customary when growing for sugar production. They claim thus to eliminate strains with an easy bolting habit and to obtain seed that will rarely bolt under ordinary conditions. On the other hand, seed raised in Poland, where late sowing followed by very uniform growing conditions is the rule, is reported to give a large proportion of bolters under English or French conditions.

BOLTING IN LEEKS AND ONIONS.

The leek behaves very similarly to the beet. The commercial strains represent a mixed population, which, however, has been so far purified that it does not bolt at all when sown in the open but only when sown under glass exceptionally early, *i.e.* in December or January. Owing to its self-fertility the ordinary process of seed saving with the leek does in effect institute pure lines. Again, the usual routine of culture begins by sowing under glass in March so that selection has already been practised and the bolters weeded out. But some strains remain in commercial seed that are capable of bolting under forcing treatment. Seed of Musselburgh leek sown on 5 January gave 5 bolters from 181 plants, no bolters when sown on 2 February or 22 February under glass, and none when sown in March in the open. The first generation saved from the non-bolters of the January sowing, when sown on 15 January gave no bolters, but in the next generation seed sown on 20 December gave 3 bolters in 87. The earlier sowing had revealed a few potential bolters which had previously escaped. The same strain of seed, a year old, gave 49 bolters out of 200 from a sowing on 15 December, 45 out of 200 from

a sowing on 29 December, 11 in 192 from a sowing on 12 January and none from a sowing in the open on 16 March. Seed saved from the non-bolters of the earliest sowing when again sown on 29 December gave no bolters among 116 plants.

The Broad Flag leek showed similar results, but the proportion of bolters was higher. There is some indication with leek seed that old seed produces more bolters than new.

TABLE V.

Bolting in Leeks.

Improved Musselburgh Leek.

	Sown under glass			Sown in open		
Family	Date of sowing	Date of planting out	No. of bolters	Date of sowing	No. of bolters	
1	15. xii	4. v	49 in 200	—	—	Year old seed
2	29. xii	4. v	45 ,, 200	—	—	,,
3	5. i	19. iv	5 ,, 181	—	—	—
4	12. i	4. v	11 ,, 192	16. iii	0 in 174	Year old seed
5	2. ii	19. iv	0 ,, 200	—	—	—
6	22. ii	13. v	0 ,, 108	17. iii	0 in 350	—
7	15. i	30. iv	0 ,, 69	17. iii	0 ,, 62	First generation from non-bolters in 3
8	29. xii	5. v	0 ,, 116	12. iii	0 ,, 42	First gen. from 1
9	20. xii	20. v	0 ,, 128	—	—	Same seed as 8 but 1 year old
10	20. xii	20. v	3 ,, 87	14. iii	0 in 38	Second gen. from 7

Broad Flag Leek.

11	15. xii	4. v	130 in 198	16. iii	5 in 171	Year old seed
12	29. xii	4. v	144 ,, 198	—	—	,,
13	12. i	4. v	105 ,, 195	—	—	,,
14	5. i	19. iv	17 ,, 94	—	—	—
15	2. ii	19. iv	32 ,, 136	17. iii	0 in 250	—
16	29. xii	4. v	0 ,, 58	12. iii	0 ,, 121	First gen. from 14

Large numbers of trials were made with onions, but a different sequence was revealed. Early sowing under glass did not induce any of the onions to bolt; the habit of growth is evidently more protracted and more definitely biennial than with *Beta*. Onions were sown in the open on different dates when it became apparent that the epoch of sowing had a marked effect upon the habit of growth and the bolting and bulb formation in the following year. Approximately one might say that sowings under glass in December, January and February when planted out gave good bulbs and no bolters in that year, and the same was true of sowings in the open in March. April sowings made as a rule poor plants which bolted in the following year, still more so sowings in May, June and July did not mature in the year of sowing and mostly

bolted in the following year. August sowings also gave plants which bolted in the following year, but September sowings for the most part gave plants which formed bulbs and did not bolt in the following year. This is true of either spring or autumn onions; all one can say is that the autumn onions when sown in the summer months produce fewer bolters than the typical spring onions sown at the same time. Table VI summarises the results obtained by sowing in different months. It shows very definitely that for practical purposes onions can be sown in heat as early as December and planted out, but that open ground sowings are unsafe after the end of March. Sowings between April and August result in no bulbs in that year and many bolters in the following year. These dates must of course be read as applying to Merton conditions of a warm light soil near sea level in the neighbourhood of London.

TABLE VI.

Bolting in Onions.

No. of bolters

	Ailsa Craig		Sutton's A 1		American Danvers Yellow		Giant Rocca	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
December*	0 in 301	—	0 in 316	—	0 in 235	—	—	—
January*	0 „ 424	—	0 „ 376	—	1 „ 219	—	—	—
March	0 „ 557	—	0 „ 335	—	0 „ 370	—	0 in 168	—
April	0 „ 289	—	0 „ 294	—	1 „ 154	—	—	—
May	0 „ 300	—	0 „ 260	—	•—	—	—	—
June	0 „ 174	All	—	—	0 in 74	All	0 in 200	110
July	0 „ 256	220	—	—	0 „ 239	220	0 „ 200	177
August	0 „ 1478	825	0 in 332	233	0 „ 1316	976	0 „ 876	120
September	0 „ 669	8	—	—	•0 „ 448	45	0 „ 213	0

* Sown under glass.

SUMMARY AND CONCLUSIONS.

The ordinary stocks of the cultivated varieties of *Beta maritima*—mangolds, sugar beet and garden beet—constitute mixed populations mainly biennial in habit but containing a certain small proportion of individuals which will "bolt" under ordinary conditions of sowing in the open. This proportion rises rapidly under conditions favourable to bolting, *e.g.* early sowing, a check to growth. Sowing under glass at the beginning of the year and transplantation into the open about April will induce upwards of 70 per cent. of the seedlings to bolt. Selection of seed for two or three generations from plants which do not bolt under

such forcing conditions gives rise to strains of seed from which the bolting tendency has been so far eliminated that none will bolt under ordinary conditions of sowing in the open or even when "forced." Though "bolting" is markedly affected by environment, by conditions of nutrition, temperature, etc. to which the plant is subjected, the individuals differ in their proneness to bolt, or in the resistance they offer to conditions that encourage bolting. These differences are shown to be genetic in origin and are inherited. Thus while the ordinary mixed stocks contain individuals which will bolt without any special stimulus and others which will not bolt even under the most forcing conditions practicable, it is possible to extract a race consisting only of the latter, which might be termed a pure biennial race. The evidence suggests that high resistance to bolting as compared with low resistance is not a simple character, but it is insufficient for the framing of a definite factorial scheme.

Leeks behave like beet, though the tendency to bolt is only revealed when specially early sowings are made in December or early January. Onions follow a different rhythm; bolting is not induced by early sowing.

From the practical point of view the experiments demonstrate that commercial stocks of seeds of mangolds, sugar beet, or garden beet, can easily be freed from strains with any susceptibility to bolting under normal conditions of growth.

RYE-WHEAT HYBRIDS FROM RECIPROCAL CROSSES.

By NINA MEISTER and N. A. TJUMJAKOFF.

(With One Plate and Four Text-figures.)

CONTENTS.

INTRODUCTION.

THE hybridisation of wheat with rye presents no special technical difficulties, as has been shown by a whole series of investigators. Beginning with the eighties of last century, Carman in America, Rimpau and Tschermak in Germany, Jesenko in Austria, etc. were successful in their attempts at artificially producing hybrids of wheat with rye. There also exist many indications as to the occurrence of natural wheat-rye F_1 hybrids in wheat sowings (Leighty, 1920; Meister, 1921; Meister and Meister, 1924). In studying the aptitude of wheat for crossing with rye, some investigators have come to the conclusion that successful crossing depends in a considerable degree on the choice of the one or the other race of these plants. Thus, Dr Jesenko, studying the different wheat races from this point of view, obtained results that were far from being identical. The differing aptitude of separate wheat races for being crossed with rye has also been established by the work of the Plant Breeding Section of the Saratov Agricultural Experiment Station (cf. Table I).

TABLE I.

Crosses	No. of pollinated flowers	No. of grains obtained	Percentage of successful crosses
Winter wheat No. 648 × "Jelissejev" rye	564	341	60·5
Winter wheat No. 329 × "Jelissejev" rye	475	17	3·6

Besides the winter wheat No. 648, which showed an exceptionally high percentage of successful fertilisations with rye, the Plant Breeding Section of the Saratov Experiment Station has at its disposal a series of other races of winter wheat, as No. 4, No. 364, No. 377, belonging to the same botanical variety as No. 648, viz. *Tr. vulgare v. erythrospermum*, which in this respect fall but little behind it. It has also been established that some of the Turkestan spring wheats, *Tr. vulgare v. graecum* of the type *rigidum*, and others, cross readily with spring rye. Owing to the ready crossability of the above-mentioned races of winter wheat with rye, as well as to their other biological peculiarities, these wheats may be used for the purpose of obtaining wheat-rye hybrids through natural hybridisation. So far the investigations of the Plant Breeding Section of the Saratov Experiment Station have shown that with properly organised special sowings the mass production of natural wheat-rye hybrids presents no difficulties. Indeed, the "manufacture" of natural wheat × rye hybrids has enabled us to conduct extensive practical work with them in the field of plant breeding, the principal purpose of the Plant Breeding Section being to secure winter-resistant and prolific varieties of winter wheat (Meister and Meister, 1924; Meister, N., 1926).

Throughout these experiments wheat was used as the maternal plant. There exists a whole series of indications in modern literature on genetics and plant breeding that in inter-specific and inter-generic crosses the choice of the pistillate parent is of exceptional importance, for it is not infrequently found that crossing takes place in one direction only, the reciprocal hybrid being never obtained. Gärtner, for instance, showed that while the cross *Mirabilis Jalapa* ♀ × *M. longiflora* ♂ is successful, the reciprocal cross *M. longiflora* ♀ × *M. Jalapa* ♂ gave no seed.

Until quite recently this was also held to be true for the wheat × rye cross, all investigators pointing out that the cross only succeeds when wheat is made the mother plant.

Dr Jesenko and Dr Firbas, however, having failed to pollinate rye with wheat pollen, suggest that in conducting experimental work with different races of wheat and rye this problem might be solved in a positive way. This suggestion was based on the fact that Tschermak succeeded in obtaining crosses which by other investigators were regarded as impossible, as for instance, *Aegilops cylindrica* ♀ × *Aegilops ovata* ♂, or *Spelta* with *Triticum dicoccoides*.

Material and Methods.

Since 1919 the Plant Breeding Section of the Saratov Experiment Station has been investigating the question of the reciprocal cross of rye ♀ × wheat ♂, but it was not until 1923 that serious work on these lines became possible. The results are given below.

The parental forms used were local, viz. "Jelissejev" rye and four lines of winter wheats, No. 4, No. 364, No. 377 and No. 684, belonging to the botanical variety *erythrospermum*.

The cross was made under field conditions where the rye plants to be used as the pistillate parent were emasculated among the other plants of the sowing. Under such conditions, the pollen shed by the rye during its flowering period fills the air and experimental work with artificial pollination is greatly impeded. In order to prevent possible accidental pollination of the emasculated flowers, castration, as well as pollination with wheat, must be performed at a time when the flowering of the rye ceases. The best moment for this operation was found to be in the early morning between 4–5 o'clock, or in the evening, immediately after sunset.

With these precautions the ears of rye, after having been emasculated and pollinated with wheat pollen, are isolated by means of paper bags.

During the course of our work 446 flowers were pollinated and 49 grains were harvested, *i.e.* 10·9 per cent. of the total number of fertilised flowers, a quite considerable proportion. The grains on sowing gave rise to typical rye plants, varying only in the shape of their ear and in their rather low fertility.

The presence of only rye characters in these plants led us to regard their hybrid origin with some scepticism. Evidently, the precautions taken in castrating the rye plants and pollinating them with wheat pollen did not exclude the possibility of rye pollen reaching the stigma of the plants under experiment at the moment when the parchment bags were removed. As rye pollen must germinate more rapidly on an allied stigma than the alien wheat pollen, the offspring obtained from the cross were typical rye plants.

The variation of the plants of the supposed F_1 generation in regard to fertility may be explained by the phenomenon commonly observed when single rye plants are transferred to a field without having at their disposal the sufficient amount of pollen necessary for setting grain (Heribert Nilsson, 1916).

The indubitable failure of our work proved, however, very instructive, as it convinced us of the impossibility of carrying out the proposed task under field conditions, even if all necessary precautions were observed. Hence in the following year, 1924, we altered our procedure. In early spring, when the shooting stage set in, about 100 rye plants were transplanted into flower pots and shifted to the station where they remained until they had reached full maturity.

Before flowering single plants were transferred to a greenhouse for emasculation, and any ears which were in bloom or near it were removed.

After emasculation and subsequent isolation by paper bags the plants were shifted to the open air where they remained for several days, until the moment of pollination. Examination of the emasculated flowers and pollination itself were carried out in the greenhouse, after which the plants with isolated ears were reared out-of-doors until harvest.

We were led to perform castration and pollination in a greenhouse by the consideration that in this way the possibility of casual pollination with the pollen of rye was completely removed. Besides, other advantages connected with work in a greenhouse became apparent in the course of our experiment, which induces us to give below a short description of the greenhouse in question. It was built in 1921 for the special purpose of serving as winter quarters for F_2 wheat-rye hybrids which, in view of their exceptional value, are usually grown in flower-pots. In order to maintain a constant temperature during the whole winter, the greenhouse was built of wood and let down into the ground to a depth of $1\frac{1}{2}$ metres, the roof consisting of the usual glass frames. Owing to its being deeply sunk into the ground, the temperature of the greenhouse during the whole summer was lower, and the humidity greater than under field conditions. This led to a uniform flowering period and enabled us to prolong considerably our work on reciprocal crossing.

Under these conditions, 844 flowers of rye were emasculated in 1924, and after having been pollinated with the pollen of the winter-wheat No. 648 produced 13 grains, viz. 1·54 per cent. of successful fertilisations. It should be noted that fertilisation actually occurs more often than these figures indicate, since, on thrashing, a considerable number of strongly grown ovaries was found besides the 13 grains. In spite of successful fertilisation development is evidently checked in many cases, resulting in grains which are not always quite formed.

An analogous phenomenon was observed by Baur in the cross *Melandrium album* ♀ × *M. noctiflorum* ♂ where, after fertilisation, the developing embryo perishes on the 8th day.

The 13 grains obtained by us from the above-mentioned cross were very thin and small, and when sown in pots only 7 of them germinated.

The young shoots were very feeble, with narrow leaves, but all belonging to the wheat type. The ear stage was reached by five plants of which only one developed normally. The ears of this plant were all sterile and in their morphological characters identical with the ears of F_1 in crosses of wheat $♀ \times$ rye $♂$.

These results prove that success depends largely on the time when the rye plants are emasculated. If the operation is performed 3–4 days before flowering, or earlier, the stigmas are checked in their development and wither. In consequence of this the flowering glumes do not separate, which greatly complicates the technics of pollination and the fertilisation comes to naught. With later castration, when the coats of the anthers are distended by the ripening process and very thin, the danger of laying them open with the forceps is very great.

Thus it appears that the most favourable moment for emasculation is the day before the beginning of flowering, when the anthers turn a pale yellow. In flowers emasculated at this moment, the stigma reaches its normal development on the 2nd–3rd day and shows the highest percentage of successful fertilisations.

It has been observed also that plants which were left for a further day after pollination in the moist air conditions of the greenhouse produced a greater number of hybrid grains than those removed on the day of pollination. This may be explained by the fact that the pollen of wheat, usually slow in germinating on the stigma of rye, germinates more rapidly under the moist conditions of the greenhouse than in the field where, with high temperature and greater dryness of the air, it is in considerable danger of desiccation.

In 1925 we carried out the same crosses on a considerably larger scale, and the results are given in Table II.

TABLE II.

Crosses	No. of maternal rye plants	No. of emasculated ears of rye	No. of pollinated flowers of rye	No. of grains obtained in F_1	Percentage of successful fertilisation
"Jelissejev" rye $♀ \times$ Wheat No. 648 $♂$	81	135	3894	96	2·46

The 96 grains obtained were sown in pots and showed 47 per cent. of germination.

The young shoots, to the number of 45, were wheat-like, though

rather feeble, the sheaths of some of them being coloured with an-
thocyan. This last feature is characteristic of the parental form of
these hybrids—the pure line wheat No. 648—which is heterozygous in
respect of the colour of the sheath.

The hybrid plants were wintered in the greenhouse and 37 were
transplanted in spring into nursery soil where their development pro-
ceeded quite normally.

We may now consider the characters of these hybrids *ex* rye ♀ ×
wheat ♂ (= reciprocal cross), and in the tables given below they are
compared with the parental forms as well as with those of the hybrids
ex wheat ♀ × rye ♂ (= direct cross).

<div align="center">CHARACTERS OF THE HYBRIDS.</div>

(1) *Vegetative characters.* Before the ear stage the F_1 hybrids of the
reciprocal crosses were examined in detail for the most important vege-
tative features: character of development, pubescence of the leaf and
sheath, shape of the ligule, etc.

<div align="center">TABLE III.</div>

Plants	General character of development	Shape of bush	The leaf				Pubescence of the leaf		
			Length	Width	Coarseness	Colour of foliage	Intensity	Character of pubescence	Coarseness
F_1 of reciprocal cross	Wheat-like	Spreading	Medium	Medium	Medium	Dark-green	Medium	Short	Medium
F_1 of direct cross	,,	,,	,,	,,	,, ●	,,	,,	,,	,,
Winter wheat No. 648	,,	,,	Long	,,	,,	,,	,,	;,	,,
Winter rye "Jelissejev"	Rye-like	,,	Medium	Broad	Tender	Glaucous	Strong or medium	Double: short and medium	,,

<div align="center">TABLE IV.</div>

Plants	Auricles				Ligula		Pubescence of leaf sheath		
	Character	Size	Cilia on auricles	Presence of anthocyan	Shape	Margin of ligula	Intensity	Character	Coarseness
F_1 of reciprocal cross	Clasping	Medium	Present	Present	Strap-shaped	Dentate	Medium	Short	Medium
F_1 of direct cross	,,	,,	,,	,,	,,	,,	,,	,,	,,
Winter wheat No. 648	,,	Long	,,	,,	Cone-shaped	,,	,,	,,	,,
Winter rye "Jelissejev"	Jutting out and clasping	Short	,,	Without anthocyan	Strap-shaped	,,	Medium and feeble	,,	,,

As may be seen from Tables III and IV the F_1 hybrids of the direct, as well as of the reciprocal crosses, exhibit during their period of growth a development typical of wheat. As regards the majority of other characters, the hybrids are also suggestive of wheat.

After the exit of the ear, however, the hybrid plants, though preserving their wheat characters, sharply change their general habit. In their height, the strong waxy bloom on culms and leaves, the pubescence of the straw under the long narrow ear, the F_1 hybrids more resemble rye. This fact has also been recorded by us for F_1 hybrids obtained from direct crosses of wheat $♀ \times$ rye $♂$ (Meister and Meister, 1924).

(2) *Flowering characters.* The data in regard to the flowering of the F_1 hybrids obtained from reciprocal crosses are given in Table V.

TABLE V.

Plants of F_1 generation of crosses	Flowering glumes during flowering	Anthers			
		Size	Texture of coat	Filaments	During flowering
"Jelissejev" rye $♀ \times$ wheat No. 648 $♂$	Open	Large	Leathery	Of wheat	Escape and without dehiscing wither
Wheat No. 648 $♀ \times$ "Jelissejev" rye $♂$	,,	,,	,,	,,	,,

Evidently the flowering of the hybrids of both crosses proceeds quite uniformly, the presence of leathery, non-dehiscent anthers entirely excluding the possibility of self-pollination. The latter fact is confirmed by the amorphous pollen found in the F_1 plants obtained from direct, as well as from reciprocal crosses.

The pollen of all hybrid plants belonging to F_1 of the reciprocal crosses was examined by us, and the results obtained are given in Table VI.

TABLE VI.

Classes according to the percentage of normal pollen...	0–1	2–3	4–5	7·4	Total number of cases
Number of cases:					
In absolute figures ...	18	6	6	1	31
In percentages ...	58	19·4	19·4	3·2	100

It is clear that a very great predominance of empty, amorphous pollen is characteristic of all F_1 hybrids of the reciprocal crosses. The inconsiderable number of normal pollen grains found in the anthers, reaching in one case up to 7·4 per cent., though not devoid of interest, cannot influence fertilisation in view of the leathery anthers. This

statement is supported by the data of Professor V. R. Zalensky, who has investigated cytologically the F_1 rye-wheat hybrids (wheat ♀ × rye ♂), and points out that the isolated normal pollen grains of these hybrids are not able even to germinate. It is obvious, therefore, that in crosses of rye with wheat the maternal form used does not influence the fertility of the F_1 hybrids, since in both crosses, the direct as well as the reciprocal one, forms are obtained incapable of self-pollination.

(3) *Fertility*. At the present time it may be affirmed that seed from the F_1 hybrids—wheat ♀ × rye ♂—can only be obtained by repeated pollination with rye or wheat (G. K. and N. Meister, 1924; Jesenko, 1913). This has also been verified by us for the F_1 plants of the reciprocal crosses.

To test the possibility of self-pollination in F_1 hybrids of reciprocal crosses, 37 ears, *i.e.* one on each plant, were isolated by us and not a single grain was harvested. When the same hybrids were pollinated with the pollen of wheat or rye, results were obtained which are given in Table VII.

TABLE VII.

Plants of F_1 generation of crosses	Year of experiments	No. of pollinated ears		No. of pollinated flowers		No. of grains obtained in F_1	
		With wheat	With rye	With wheat	With rye	With wheat	With rye
"Jelissejev" rye ♀ × wheat No. 648 ♂	1926	27	7	1022	294	12	0
Wheat No. 648 ♀ × "Jelissejev" rye ♂	1919	50	50	1004	992	6	1

Since we had no F_1 plants from direct crosses in our 1926 sowings, the data given in the above table are compared with the results of direct crossing in 1919.

Nevertheless, the F_1 plants of both crosses are characterised as forms identical in their fitness for artificial repeated pollination with wheat or rye.

TABLE VIII.

	No. of grains													
Plants of F_1 generation of crosses	1919		1920		1922		1923		1924		1925		1926	
	Per ear	Per plant	Per ear	Per plant	Per ear	Per plant	Per ear	Per plant	Per ear	Per plant	Per ear	Per plant	Per ear	Per plant
"Jelissejev" rye ♀ × wheat No. 648 ♂	—	—	—	—	—	—	—	—	—	—	—	—	0·30	0·84
Wheat No. 648 ♀ × "Jelissejev" rye ♂	0·17	—	0·07	0·43	0·12	0·45	0·12	0·40	0·27	1·2	0·11	1·42	—	—

The fertility of non-isolated F_1 ears through natural pollination by wheat or rye is indicated in Table VIII, which shows that the fertility of both classes of F_1 hybrids is approximately the same.

(4) *Ear characters*. The general habit of the ears borne by the F_1 plants of both crosses is similar. They are characterised by long narrow ears and rather flexible awns, straight, but adpressed to the ear. The straw at the base of the ear is pubescent (Plate IV).
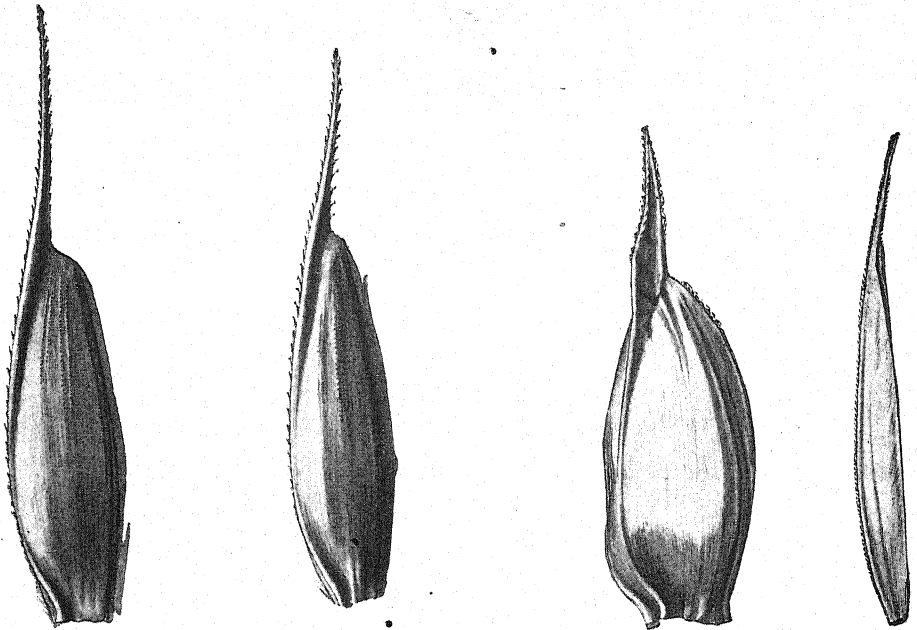

Fig. 1.   Fig 2.

Fig. 1. Empty glumes of F_1 hybrids, from left to right, of reciprocal and direct crosses.
Fig. 2. Empty glumes of wheat No. 648 and of "Jelissejev" rye.
(Drawings by V. Pashine.)

In the number of spikelets and the average length of the ear, the F_1 hybrids of both crosses approach rye. As regards the empty glume and the density of the ear, they are in both cases more suggestive of wheat, as may be seen from Table IX.

Preserving its wheat type, the empty glume of the F_1 hybrids of both crosses is somewhat elongated and narrowed if compared with the parental form—winter wheat (pure line No. 648)—as illustrated in Figs. 1 and 2.

TABLE IX.

Plants	Length of ear in cm.		No. of spikelets		Density of ear		Relation of width to length in empty glume	
	Average	Fluctuations	Average	Fluctuations	Average	Fluctuations	Average	Fluctuations
Winter rye "Jelissejev"	11·0	6·0–14·5	33	24–42	3·12	2·6–3·8	9·9	8·0–14·3
F_1 of "Jelissejev" rye ♀ × wheat No. 648 ♂	12·5	7·7–17·2	24	18–30	2·0	1·5–3·2	3·7	3·2– 4·3
F_1 of wheat No. 648 ♀ × "Jelissejev" rye ♂	11·0	6·5–14·0	24	13–31	2·3	1·9–3·1	4·5	3·3– 6·7
Winter wheat No. 648	8·0	7·0–10·0	16	14–19	2·0	1·7–2·5	3·0	2·6– 3·6
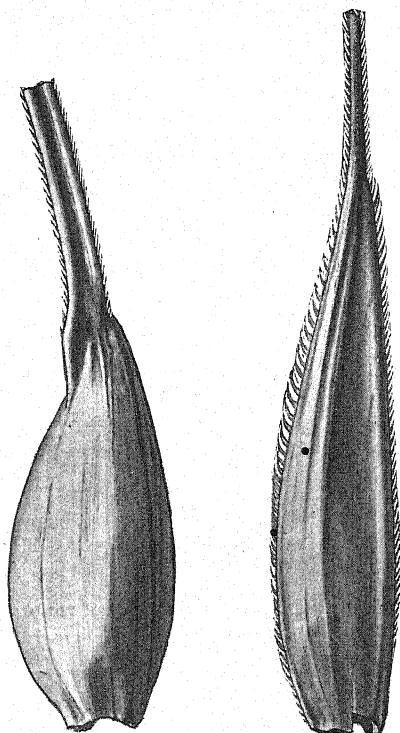
Fig. 3. Outer flowering glumes of wheat No. 648 and of "Jelissejev" rye. (Drawing by V. Pashine.)

Before describing the outer flowering glume of the F_1 hybrids, we may allude briefly to the peculiarities of this structure in the parental forms, viz. winter wheat No. 648 and "Jelissejev" winter rye.

In wheat the outer flowering glume is somewhat oval; the awn,

markedly thickened below, shows an inflated base by which it is attached to the summit of the flowering glume.

In rye, the outer flowering glume is strongly elongated and lanceolate. The central nerve forms a sharply marked keel throughout the whole length from tip to base. This keel is toothed or scabrous, a feature which together with the keel itself is not found in wheat (Fig. 3).

The outer flowering glume of the rye-wheat hybrids is intermediate in shape between wheat and rye. The awn, where it is attached to the
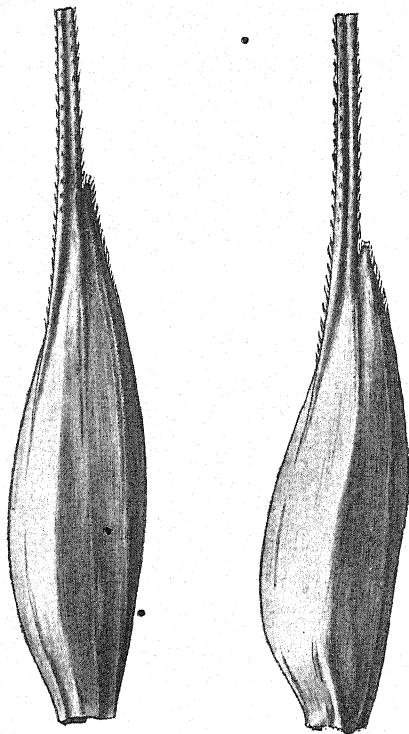
Fig. 4. Outer flowering glumes of the F_1 hybrids, from left to right, of reciprocal and direct crosses.   (Drawing by V. Pashine.)

glume, is not inflated. The keel, forming as if in continuation of the awn and marked only in the upper part of the glume, bears minute spines (Fig. 4).

It is clear, therefore, that crosses between wheat and rye, no matter whether the pistillate parent be wheat or rye, give rise to F_1 hybrids which are perfectly identical in development and fertility, as well as in their morphological characters. Consequently, there can be no question

of maternal inheritance in the F_1 hybrids obtained from crosses of rye with wheat.

CONCLUSION.

In conclusion we wish to mention the paper of E. F. Gaines and .F. J. Stevenson, published in 1922, and treating of F_1 and F_2 hybrids obtained from crosses of rye ♀ × wheat ♂. The authors state that the F_1 plants of the reciprocal crosses were characterised by a predominance of rye-characters, the fertility of the ears varying from 50 to 90 per cent. The crosses carried out by us in 1924 and 1925, however, have led us to another, quite definite cônclusion, namely, that the F_1 hybrids from reciprocal crosses carry no rye-characters in them, but resemble *Triticum*.

Further, these authors state that they obtained in the F_2 generation exclusively plants of a rye-like character, which is illustrated by the photographs reproduced in their paper. Though the F_2 hybrids of reciprocal crosses have not yet been investigated by us, there seems little probability of obtaining such plants.

Seeing that the F_1 zygote carries 21 wheat chromosomes as against 7 rye chromosomes, we certainly ought to have more chances of obtaining a wheat type after segregation.

In our direct crosses, at any rate, when several thousands of plants belonging to the F_2 generation were analysed, in two cases only were plants of the genus *Secale* observed, and it must be said that we are not quite certain as to the hybrid origin of these forms. With more or less probability this may be affirmed only for one plant which deviated markedly from common rye by the three times broader foliage of the autumnal bushes.

SUMMARY.

1. Crosses between wheat and rye present no special difficulties if wheat is used as the mother plant. The percentage of successful fertilisations depends in this case chiefly on the choice of the races intended for crossing purposes.

2. Reciprocal crosses, where the pistillate parent is rye, were until quite recently considered impossible. We succeeded, however, in making such crosses when the flowers of local winter rye were pollinated with the pollen of the pure line wheat No. 648, which readily hybridises with rye in direct crosses.

3. In 1925, when the technique of these crosses had been sufficiently elaborated, we obtained 96 grains from 3894 rye flowers emasculated
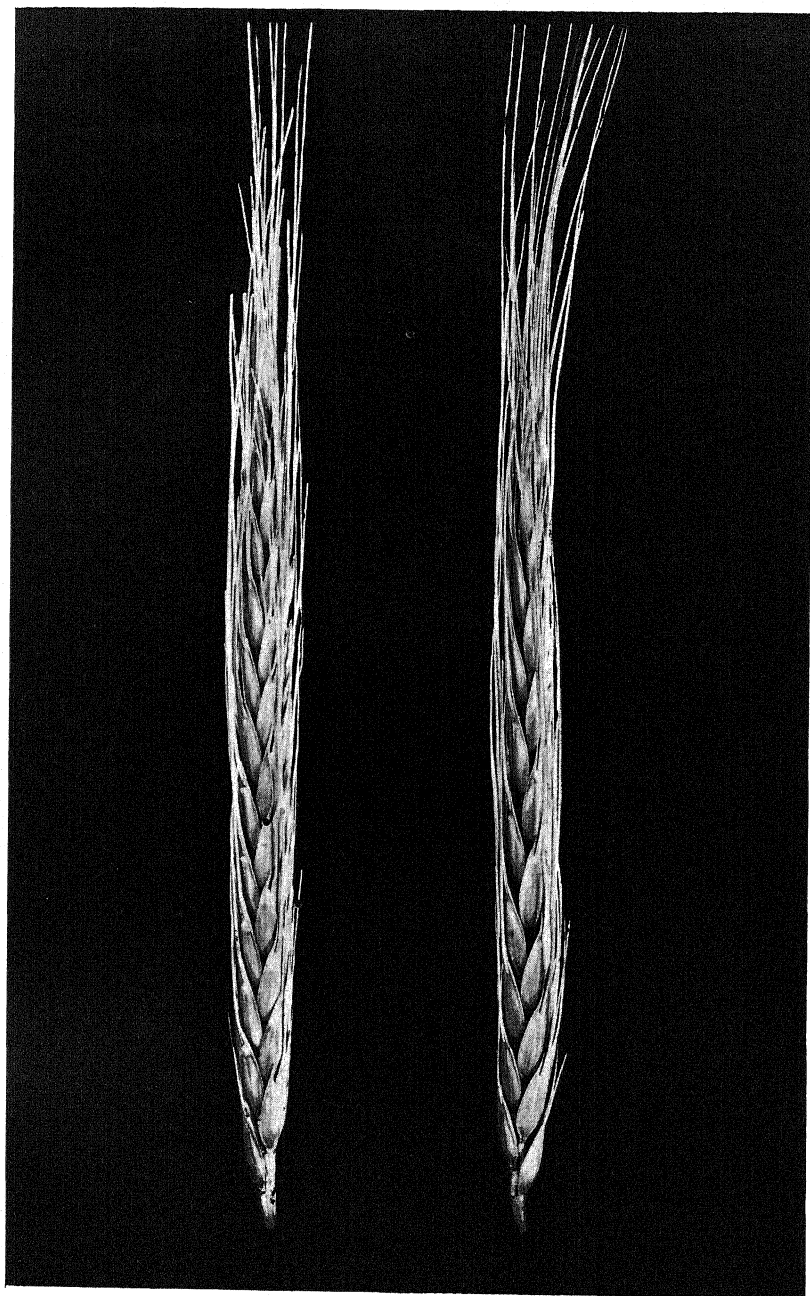
and pollinated with wheat pollen, *i.e.* 2·5 per cent. of successful fertilisations, while in direct crosses this percentage usually exceeds 60.

4. F_1 plants from direct and reciprocal crosses proved to be identical in morphological characters, as well as in fertility.

5. Maternal inheritance recorded by E. F. Gaines and F. J. Stevenson for reciprocal crosses—rye ♀ × wheat ♂—has not been confirmed by our experiments.

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EXPLANATION OF PLATE IV.

Ears of F_1 hybrids, from left to right, of direct and reciprocal crosses.
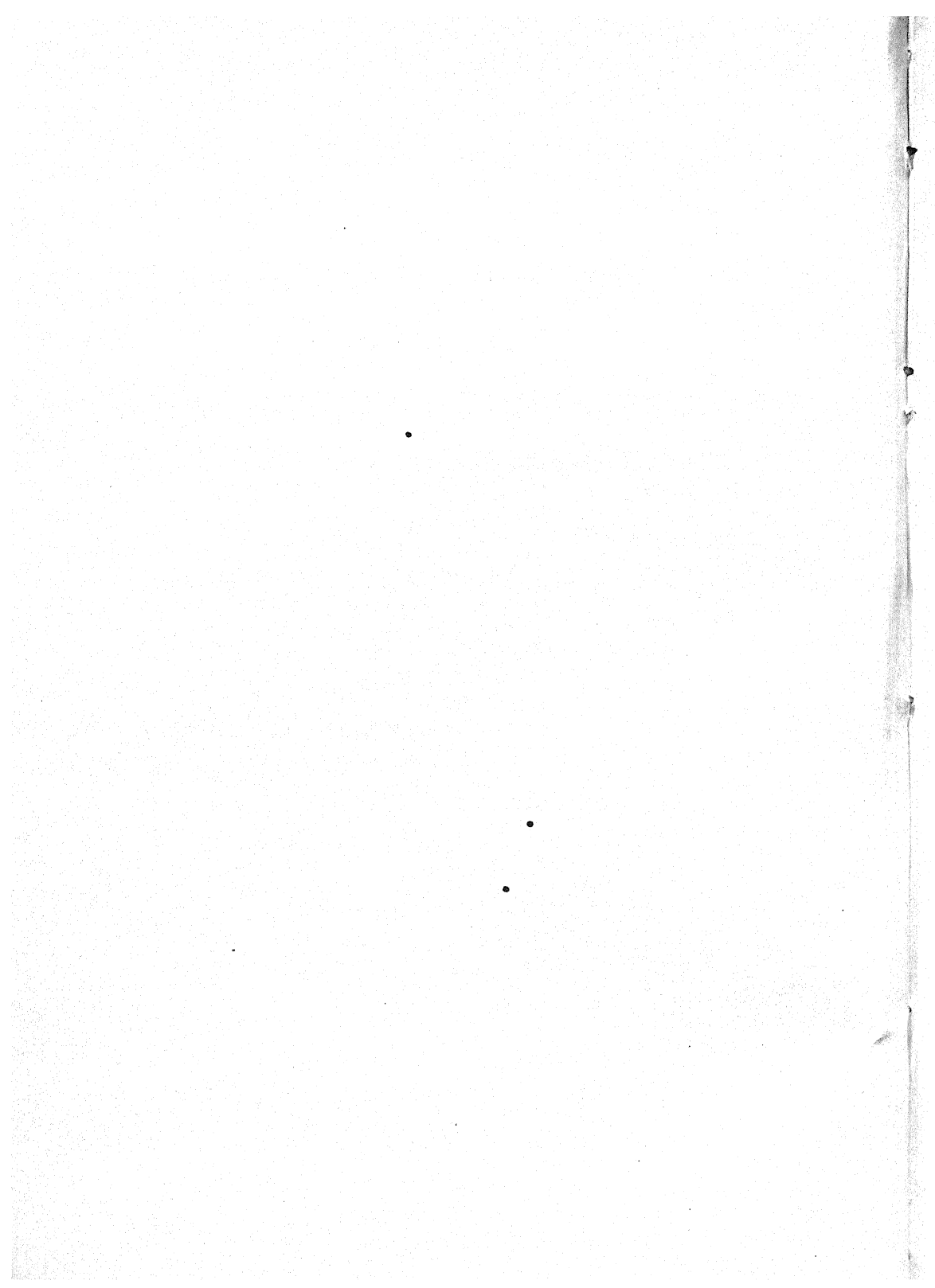
FURTHER NOTES ON DUTCH AND ENGLISH RABBITS.

By R. C. PUNNETT, F.R.S.

(With Six Text-figures.)

THIS is a further contribution towards the settlement of the difference between Prof. Castle and myself as to the genetical interpretation of the various grades of Dutch rabbits and of their relation to the self-coloured animal. As our controversy has been in abeyance for nearly three years I may perhaps be forgiven if, before considering the fresh data, I recall briefly how the case stands.

The extent of recessive white markings in rabbits, of which a particular distribution characterises the Dutch pattern of the Fancy, shows an enormous range of variation from animals which are not far from self-coloured to animals in which the pigment is reduced to the merest trace. Between these extremes every grade of pigmentation is to be found (cf. Fig. 1), and the problem before the geneticist is whether such a continuous series, together with the results that follow when any members of it are crossed together, can be expressed on the usual factorial system. For the past 20 years or so Prof. Castle and I have between us bred thousands of Dutch-marked rabbits, and we have both come to the conclusion that the great range of variation shown can be expressed in terms of relatively few genetical factors. Our respective interpretations however are not quite the same, and in order to bring out the essential difference I may present briefly my own view and then compare it with that of Castle.

Originally set out in 1920, and subsequently confirmed by a series of experiments published by Mr M. S. Pease and myself five years later, this solution postulates one "major" factor and three "minor" factors, though only two of these were met with in our own work. It is presented graphically in Fig. 2. The lowest grade of pigmentation (1a) lacks both the major factor, **P**, as well as the two minor factors, **S** and **T**, and is constitutionally **ppsstt**. With either **S** or **T** the amount of pigment increases, and the increase is greater in the homozygous than in the heterozygous condition. Additional doses of either **S** or **T**, or both, account for the grades shown as 2a—4a, until when both **S** and **T** are present in the homozygous condition the grade of pigmentation is raised
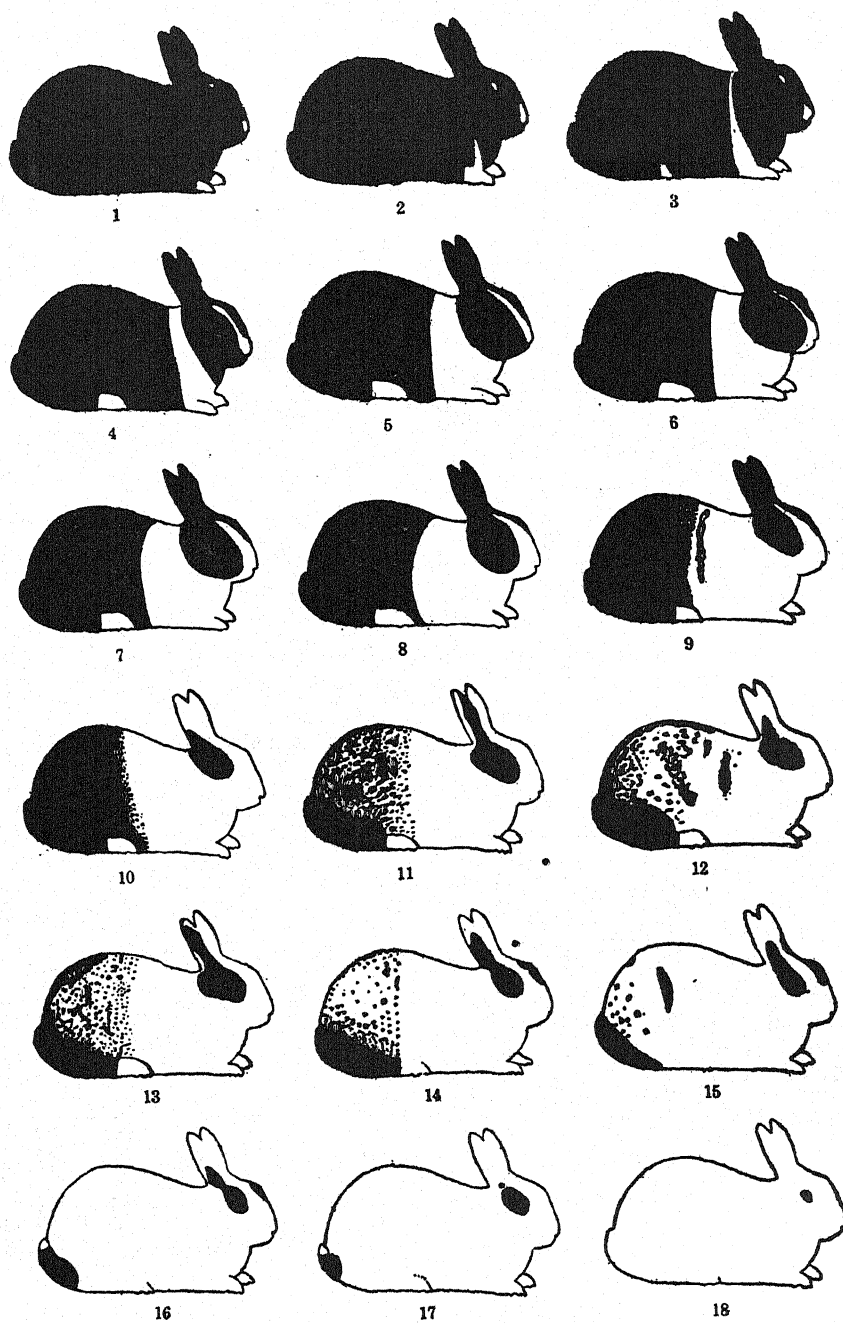
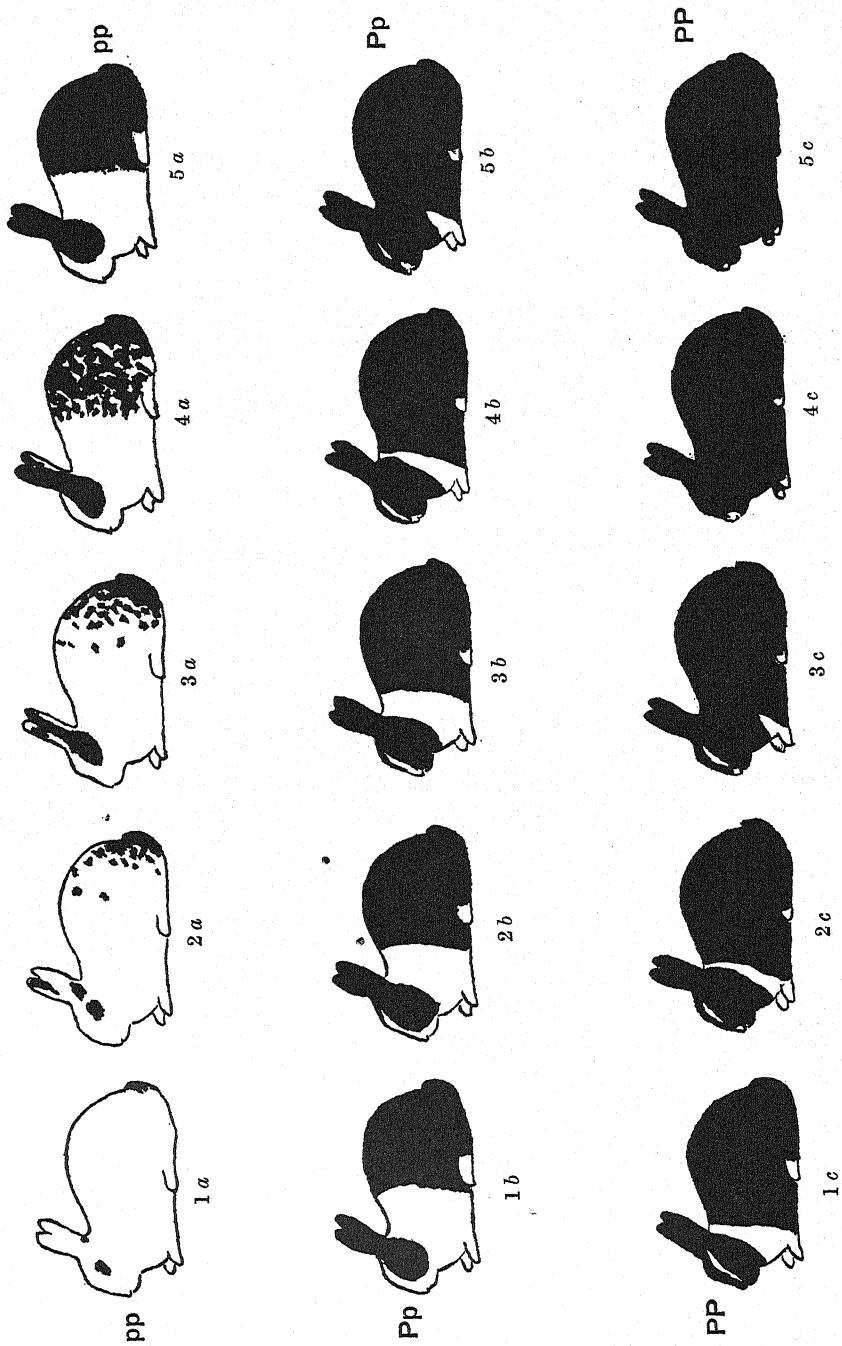Fig. 1. Grades 1–18 of Dutch rabbits (from Castle).

Fig. 2.

to that of the typical Dutch of the Fancy (5a). Higher grades of pigmentation are brought about by the "major" factor, P^1, which also produces a greater effect in the homozygous than in the heterozygous state. The two lower lines in Fig. 2 indicate the effect produced on the members of the basal series 1a—5a when the animal becomes heterozygous for **P** (1b—5b) or homozygous (1c—5c). A rabbit homozygous for **P**, **S**, and **T** is very nearly or completely self-coloured[2]. Probably however another "minor" factor, N^3, similar in its operation to **S** and **T**, also occurs in most self-coloured breeds. The gist of this interpretation is that it explains the white-marked series, from the whitest grade of Dutch up to and including the completely self-coloured animal, in terms of four factors, of which one, **P**, may be regarded as a major factor, and the other three as minor or modifying factors.

The main point of difference between this interpretation and that of Prof. Castle is that he regards self-colour as a thing *per se*, and in place of our factor **P** postulates a series of three multiple allelomorphs, viz. **Du** for self-colour, **du$_d$** for "Dark Dutch" with a considerable amount of pigment, and **du$_w$** for "White Dutch" with a smaller amount of pigment. He admits the existence of modifying factors but has not attempted any analysis of them, and in this respect his interpretation must necessarily lack precision. Elsewhere[4] I have pointed out that Castle's scheme does not cover his own data so well as the one that I have suggested. Moreover it breaks down definitely in the case of one of his crosses between Dark Dutch (**du$_d$**) and White Dutch (**du$_w$**), since a considerable proportion of self-coloured animals appeared both in F_1 and F_2[5]. The formation of the most dominant member of a series of multiple allelomorphs from a cross between two of the recessive ones is a striking phenomenon, and to most geneticists would at once suggest that there was something wrong with the scheme of interpretation.

More recently (1926) Castle has advanced further evidence from which he claims support for his hypothesis. A few years previously he

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[1] A distinctive feature of **P** is that when present it prevents the eyes from showing any *heterochromia iridis*, but any rabbit without **P** may exhibit *heterochromia*.

[2] As recorded in our 1926 paper Mr Pease and I had not then found a **PPSSTT** animal entirely devoid of white hairs, though these might consist of three or four hairs only on the tip of the nose or on a fore paw. Since then however we have bred some which were completely self-coloured even under the most critical examination.

[3] Cf. *Journ. Gen.* 1925, xv. 397.

[4] *Journ. Gen.* 1920, ix.

[5] *Journ. Gen.* 1926, xvi. 198.

had made the interesting discovery that Dutch pattern showed linkage with the long Angora hair, and he has now made ingenious use of this phenomenon in an attempt to demonstrate the existence of his three Dutch allelomorphs. In doing so he has brought together a fine body of experimental data demanding careful consideration. Of the particular experiments in which we are here interested the starting-point was a typical Dutch ♂ (Fig. 1, 8) which had been proved to carry White Dutch[1], and therefore on Castle's hypothesis du_ddu_w. Mated with self-coloured Angoras (**DuDu**) he gave two classes of young, viz. **Dudu$_d$** and **Dudu$_w$**, the latter class being visibly distinct from the former in having more white. Since in each case normal short hair (**L**) goes into the cross with Dutch, and Angora hair (**l**) with self-colour, we should expect F_1 animals so produced to form an excess of **Dul** and of either **du$_d$L** or **du$_w$L** gametes, as the case may be. In other words Angora hair should be associated generally with a higher grade of pigmentation than short hair. Several of the F_1 animals with more white, and of the presumed constitution **Duldu$_w$L**, were mated back to doubly recessive White Dutch Angora ♀♀, **du$_w$ldu$_w$l**, and as is quite clear from Tables XI–XV in Castle's paper there is linkage between Angora hair and darker pattern, and between short hair and lighter pattern[2]. For Castle this linkage is evidence for the allelomorphic nature of **Du** and **du$_w$**; on my alternative hypothesis it means that there is linkage between **P**, which was carried by the self-coloured rabbit, and **l**.

The other type of F_1, resulting from the "Dark Dutch" gamete of the Dutch ♂ and therefore constitutionally **Duldu$_d$L**, was also mated with the doubly recessive White Dutch Angora. In this case F_1 ♀♀ were mated with White Dutch Angora ♂♂. The essential data form part of Table XVII (p. 29) in Castle's paper, and as they are of great importance for our discussion I reproduce them here in slightly modified form:

	Grade of Angora young											Total	Average grade
Nature of mating	0	1	2	3	4	5	6	7	8	9	10		
♂ 5990, du$_w$ldu$_w$l × ♀♀ Duldu$_d$L	1	7	11	10	3	1	—	—	—	—	—	33	2·30
♂ 5941 ,, × ,,	7	11	25	18	14	5	—	—	—	1	—	81	2·53
♂ 6248 ,, × ,,	—	2	2	5	2	5	1	1	—	1	—	19	4·00
Totals	8	20	38	33	19	11	1	1	—	2	—	133	2·68

[1] Such an animal is what we have termed "Mock Dutch" (cf. Punnett and Pease, 1925, p. 386) and genetically is usually **Ppsstt**, though it may be heterozygous for either **S** or **T**.

[2] The data from Table XII of Castle's paper are shown graphically in the upper part of Fig. 3 of this present paper (p. 253).

Nature of mating	Grade of Short-haired young											Total	Average grade
	0	1	2	3	4	5	6	7	8	9	10		
♂ 5990, du_wldu_wl × ♀♀ $Duldu_dL$	—	1	3	5	3	4	—	—	—	—	—	16	3·37
♂ 5941 ,, × ,,	3	6	7	21	8	6	4	2	—	—	—	57	3·21
♂ 6248 ,, × ,,	—	—	1	5	4	2	—	1	—	2	1	16	4·87
Totals	3	7	11	31	15	12	4	3	—	2	1	89	3·54

It will be noticed that the figures for both Angora and short-haired show a similar distribution when arranged in Castle's pigmentation grades, a point brought out more clearly in the graphical representation in the lower part of Fig. 3 (p. 253 of this paper). In neither case is there any hint of the bimodal distribution which was so striking in the data demonstrating linkage between the Self-White and Dutch and the long and short-haired pairs (cf. Fig. 3, upper part). There is no marked preponderance of the darkest animals among the Angoras or of the lightest among the short-haired as would be expected from the nature of the mating if **Du** and **du$_d$** behaved as allelomorphs. Nevertheless the Angoras as a whole are of slightly darker grade than the shorts, averaging 2·68 as against 3·54—a difference of ·86 grade. How much this amounts to can be inferred from Castle's table of grades here reproduced as Fig. 1 (p. 248). This difference is regarded by Castle as sufficient to establish the existence of linkage between his two postulated allelomorphs, **Du** and **du$_d$**, and the short Angora pair. For the following two reasons however I do not consider that the case for linkage has been made out.

(1) If we were concerned with linkage we should expect a much greater difference in the average grades of the Angora and short-haired classes. Since the cross is presumed to be between F_1 animals of the constitution **Dudu$_d$** and White Dutch, **du$_w$du$_w$**, the two types **Dudu$_w$** and **du$_d$du$_w$** must occur in equal numbers. Now Castle has already stated that in his material the average grade of **Dudu$_w$** animals is 1·51 (1919, Table XXVII, p. 44), while that of **du$_d$du$_w$** animals is 7·28 (1919, Table XXII, p. 43)—a difference in mean grade of 5·77. Assuming linkage with about 14 per cent. of cross-overs this difference would be somewhat reduced, since about 1 out of every 7 Angoras would be associated with **du$_d$** instead of **Du**, and the same proportion of shorts would be linked with **Du** instead of **du$_d$**. Hence our Angora mean grade would become $\dfrac{1·51 \times 6 + 7·28}{7} = 2·33$ instead of 1·51, and the mean grade of the shorts would similarly be shifted to $\dfrac{7·28 \times 6 + 1·51}{7} = 6·45$. In other words we
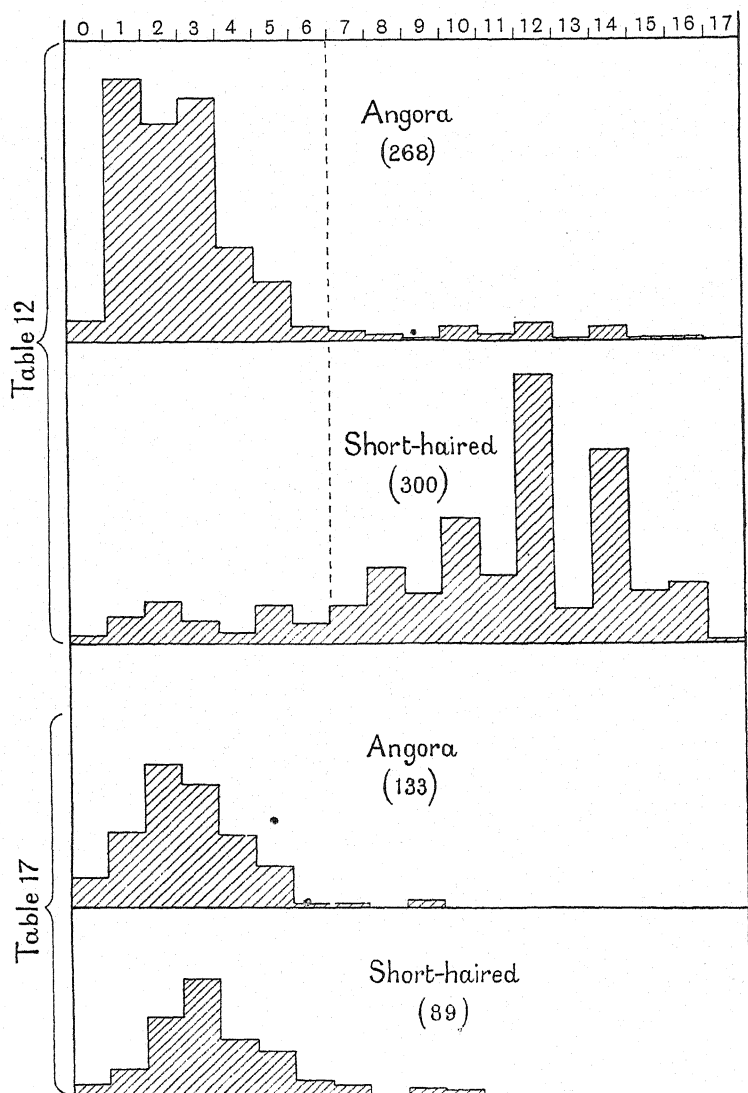
Fig. 3. A graphic representation of the data given in Tables XII and XVII of Castle's paper. The upper part of the figure shows the distribution of the grades of pigmentation on the Angoras and short-haired respectively when F_1 rabbits heterozygous for Self and White Dutch as well as for long and short hair were mated back with the double recessive, viz. White Dutch Angora. The distribution of the Angoras and short-haired among the 568 animals clearly indicates linkage. Similar results were also given by the data in Tables XI, XIII, XIV and XV.

The lower part of the figure shows the distribution when F_1 rabbits heterozygous according to Castle for Self and Dark Dutch as well as for long and short hair were similarly mated back with White Dutch Angora.

should expect a mean grade difference of 4·12 between the Angora and short classes in this experiment if linkage with factors for self and Dark Dutch pattern occurred. That the mean grade difference is only ·86 seems to me to tell definitely against the existence of any linkage here.

(2) Still, the mean grade difference of ·86 between the Angoras and shorts is evidently a definite phenomenon in this set of experiments, and the question is whether we can account for it. Castle has laid stress on the fact that his method of recording the grade of pattern before the nature of the hair is to be distinguished obviates any possibility of unconscious bias in grading the young, nor have I any intention of questioning the accuracy of his attributions. The explanation I wish to suggest is that although the Angora cannot be definitely detected by ordinary inspection until several weeks after birth, nevertheless its hair growth must be different from the earliest stages, and it may well be that this structural difference leads to the pigment spreading more, as it were, and so bringing the Angora up to a slightly higher grade than the short-haired animal of corresponding genetical constitution. With this idea I have gone over the data given in Tables XI–XIV of Castle's paper in which the material for the $\mathbf{Dudu_w} \times \mathbf{du_w du_w}$ cross is closely comparable to that for the $\mathbf{Dudu_d} \times \mathbf{du_w du_w}$ cross under discussion. In each case the mean grade of Angoras and shorts has been calculated separately for both the $\mathbf{Dud_w}$ and $\mathbf{du_w du_w}$ classes of progeny, with the results set out below:

Table	Total no.	Angora $\mathbf{Dudu_w}$	Short $\mathbf{Dudu_w}$	Difference	Angora $\mathbf{du_w du_w}$	Short $\mathbf{du_w du_w}$	Difference
XI	167	2·65	3·23	+ ·58	9·88	10·39	+·51
XII	568	2·39	3·16	+ ·77	11·16	11·87	+·71
XIII	170	4·24	5·36	+1·12	13·50	14·11	+·61
XIV	53	3·60	3·66	+ ·06	13·50	13·21	−·29

In the first three experiments (Tables XI–XIII) involving over 900 animals the Angora class is consistently more pigmented than the corresponding short-haired class. Only in the fourth experiment (Table XIV) is there a slight difference the other way, but the numbers here are relatively so small that a few exceptional animals in either class may quite well have turned the scale, and I cannot think that this case is of comparable significance to the other three cases (Tables XI–XIII) where the numbers are so very much larger. Should later work confirm the conjecture that the Dutch Angora is normally a little more pigmented than the corresponding short-haired animal this would account for the difference of ·86 grade which Castle claims as evidence of linkage,

and so of any genetical difference between his postulated allelomorphs **Du** and **du$_d$**.

I need hardly add that the absence of linkage phenomena in this last case is what is to be expected on the alternative hypothesis. For on this view Castle's **Du** and **du$_d$** are essentially one and the same thing, viz. **P**; and the cross in question was of the nature **PPLl × ppll**. Linkage phenomena between **P** and **L** would of course only occur where an animal is heterozygous for both factors, as in the experiments recorded in Castle's Tables XI–XV. In an earlier criticism (1926, p. 199) I suggested that Prof. Castle could readily bring into line our rival views by identifying his **du$_d$** factor with **P**, his **du$_w$** factor with **p**, and by scrapping his **Du** factor altogether. This suggestion, after careful consideration of the additional evidence presented in his 1926 paper, I see no reason to alter.

ON THE RELATION BETWEEN DUTCH AND ENGLISH.

Some years ago Castle made the interesting discovery that there exists a close linkage between the English pattern and the Dutch. English × Dutch gives English F_1 and an F_2 generation consisting of English and Dutch in the ratio 3 : 1, except for the very rare cross-overs. These of course are of two kinds, viz. English-Dutch, in which the English inhibitory factor is superposed as it were upon the Dutch pattern, and self-colour lacking both English and Dutch factors. Hitherto Castle has only obtained an example of the former (1926, p. 31) owing doubtless to the extreme closeness of the linkage. Nevertheless his experiments, by bringing in also the short-hair Angora pair, have thoroughly established the existence of the linkage. Now the English rabbit is genetically a self-coloured animal *plus* the inhibitory factor (**I**) which brings about the dominant English pattern, and it occurred to me that the existence of the linkage discovered by Castle might be used to throw further light upon the nature of the Dutch pattern. On the view that I have suggested the English rabbit is to be regarded as **IIPP** together with certain minor factors which, in combination with **P**, serve to bring up the grade of pigmentation to self-colour. Since these minor factors (*e.g.* **S**, **T**) cannot be satisfactorily determined without independent analysis, and since different races of self-coloured rabbits may differ in respect of them[1], we may for the present speak of them collectively as **X**, using **X$_1$**, **X$_2$**, **X$_3$** to denote the different individual factors where required. If now an English rabbit (= **IIPPXX**) be crossed with a White Dutch of the lowest

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[1] Cf. *Journ. Gen.* 1925, xv. 397.

grade (= **iippxx**) the F_1 animals will be constitutionally **IiPpXx**, and if **I** and **P** be very closely linked the gametes will be practically all of the four types **IPX, IPx, ipX, ipx**, the last two types alone giving rise to Dutch offspring. But since **X** stands for certainly more than one, probably three, possibly even four minor factors, the Dutch offspring, provided that enough are raised, should exhibit a considerable range, viz. from $x_1x_1x_2x_2x_3x_3...$ up to $X_1X_1X_2X_2X_3X_3....$ Further, there is the possibility that *heterochromia iridis* may show itself in any Dutch animal so bred though it should be found in none of the English[1].
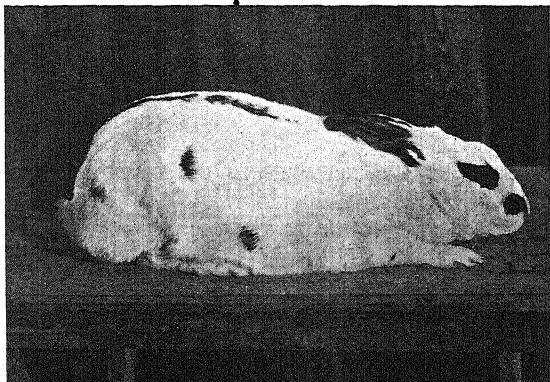
Fig. 4. ♀ S 214.

To test the point I purchased in 1925 a black English doe (S 204) of the very light form known as a "Charlie Chaplin," and mated her to U 81, a black White Dutch ♂ (**iippxx**)[2]. Unfortunately she produced only a single daughter, S 214, similar to herself (cf. Fig. 4) and died before a further litter could be obtained. S 214 produced 4 litters to her father and 1 to the tortoise White Dutch ♂ A 82. From one of these matings a black English doe, B 45, very like her mother (cf. Fig. 5) was kept and also mated to U 81 and A 82. The relations of the different animals used are shown in the accompanying pedigree:

$$\text{♀ S 204} \times \text{♂ U 81}$$

$$\text{♂ A 82} \times \text{♀ S 214} \times \text{♂ U 81}$$

$$\text{♂ A 82} \times \quad \text{♀ B 45} \times \text{♂ U 81}$$

$$\text{♂ A 82} \times \text{♀ B 70}$$
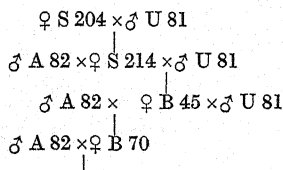
Excepting of course the very rare cross-over form.
On our ordinary nomenclature he was **ppsstt**.

Eight litters in all were bred from S 214 and B 45 when mated to the two **iippxx** ♂♂ U 81 and A 82. The young were all either English or Dutch, and in respect of colour either black or tortoise. The English were all on the light side, none having as much pigment as the typical English of the Fancy, nor was any one of them heterochromic.
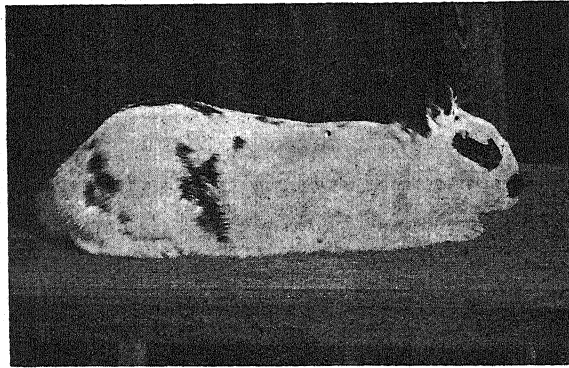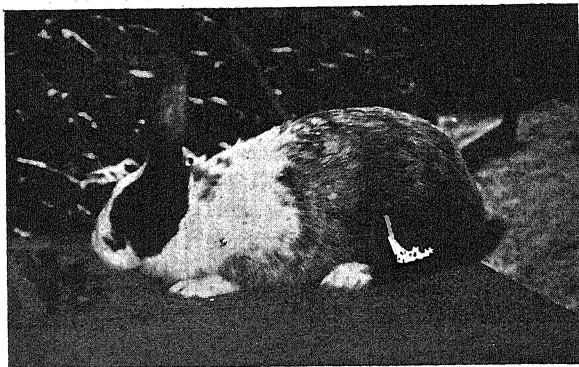

Fig. 5. ♀ B 45

Fig. 6. ♀ B 70.

The 43 young being distributed among the four classes as follows:

Black English	14
Tortoise English	6
Black Dutch	14
Tortoise Dutch	9

The interesting feature is the nature of the Dutch offspring. Of these 14 were White Dutch or near White Dutch (cf. Fig. 2, 1a—2a), 8 varied

fairly closely round grade 3*a* in Fig. 2, and one, B 70, was distinctly more pigmented (cf. Fig. 6). These animals were of course all back-crosses with White Dutch containing no "minor" factors, and in translating them into terms of the gametic output of the English mothers these grades must be raised throughout, except in the case of the White Dutch. It is likely that B 70 for example received three minor factors from her mother, and that the homozygous type to which she corresponds is probably not far removed from Castle's "Tan Dutch" (cf. Fig. 1, 4). At the other end of the scale we have White Dutch almost entirely lacking in pigment, which can have received no minor factors from their mother. Though the distribution of the different grades accords reasonably well with the hypothesis that three minor factors are concerned, a far greater number of experiments would be required for a satisfactory analysis. Nevertheless the data serve to demonstrate the point that from a cross between English and White Dutch the Dutch series in F_2 (as judged by the back-cross) shows a range corresponding to the minor factors affecting the Dutch pattern which were present in the English, and to these alone.

Should Prof. Castle still be anxious to demonstrate the existence of a definite allelomorph for self-colour I venture to suggest the following experiment. Let him take such F_1 English × White Dutch[1] as I have used and back-cross the English offspring with White Dutch for several generations, in this way ridding his English of all "minor" modifying factors. Then let him continue to cross the English thus purified of modifying factors with White Dutch until the rare cross-over "self" gamete (**Du**) is met with. From such an animal he should be able, if his hypothesis be true, to obtain eventually a strain of selfs which, when crossed with White Dutch, will give only three definite types in F_2, viz. selfs (**DuDu**), White Dutch (**du_w du_w**), and the heterozygous form (**Dudu_w**) in the ratio 1 : 1 : 2.

A PROBABLE CASE OF LINKAGE.

In connection with these few experiments there has arisen a further point of interest concerning the progeny of the three does S 214, B 45 and B 70, all of which are presumably heterozygous for the several minor modifying factors that existed in the original English ♀ S 204. Since they were all heterozygous for black their offspring from the tortoise White Dutch ♂ were blacks and tortoises in approximately

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[1] It is understood of course that "White Dutch" is here used in my sense, signifying an animal not more pigmented than Castle's grade 17.

equal numbers. When the offspring were graded it was found that the majority of the blacks belonged to the more pigmented classes, and the majority of the tortoises to the less pigmented. The grading was approximately[1] that made use of in an earlier paper (1925, *q.v.*), viz. into the four grades S.D., R.S.D., R.S.D.–V.R.S.D., V.R.S.D. in order of decreasing amount of pigmentation, and gave the following result.

	S.D.		R.S.D.		$\dfrac{\text{R.S.D.}}{\text{V.R.S.D.}}$		V.R.S.D.	
	Black	Tortoise	Black	Tortoise	Black	Tortoise	Black	Tortoise
S 214	—	—	—	—	—	—	1	2
B 45	—	—	—	.	—	—	2	2
B 70	1	1	5	—	2	3	1	7
Totals	2	1	5	—	2	3	4	11

From these figures it is clear that black predominates in the more highly pigmented grades and tortoise in the less pigmented. The relation is perhaps more clearly brought out in the following correlation table made by running together the two higher grades of pigmentation (S.D. and R.S.D.) as opposed to the two lower ones.

	Black	Tortoise
Higher grades	7	1
Lower grades	6	14

It should be stated that the predominance of lower grades among the tortoises is not due to the pigmented area being normally less developed than in the blacks, because in one case a tortoise young was produced by B 70 which was as highly pigmented as B 70 herself. The obvious explanation of this correlation is that there is linkage between black and one of the pigmentation factors, though which one is concerned it is of course impossible to say[2]. Few as the data are I have placed them on record because the point may be helpful to anyone who

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[1] The grading was not more than approximate since the material here was not quite the same. The classes were on the whole rather more pigmented than those in the 1926 paper. They correspond roughly to figures 1*a*—4*a* in Fig. 2 (p. 249).

[2] The existence of such a linkage is supported by results obtained in F_2 from the cross Typical Dutch (**ppSSTT**) × Deep Dutch (**PPsstt**) (cf. Punnett and Pease, 1925, p. 388). Here the Typical Dutch parents were black and the Deep Dutch were tortoise. The minor modifying factors concerned both went into the cross with black, and consequently if either of them showed linkage we should expect to find the blacks as a group showing a rather higher grade of pigmentation than the tortoises. This was actually the case, especially noticeable being the relative preponderance of tortoises among the lightest classes. We did not attach much significance to this at the time but in the light of the experiments recorded above it would receive a simple explanation on the hypothesis that either **S** or **T** is linked with the factor for black.

is tempted to take up the further analysis of the various modifying factors that enter into the complex of the self-coloured rabbit.

The expenses of these experiments were in part defrayed by grants. from the Government Grant Committee administered by the Royal Society.

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EXPERIMENTS ON THE INHERITANCE OF WEIGHT IN RABBITS.

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(With Twenty-four Text-figures.)

CONTENTS.

1. INTRODUCTION.

In 1918 Punnett and Bailey published an account of experiments on the inheritance of weight in rabbits, experiments which were of necessity preliminary, as there had not been time to make a study of the variability of the parent stocks used. A most unexpected result however emerged, namely, the non-appearance of the heavier classes of animal in F_2 generations of 18 and 47 rabbits. This non-appearance was definitely marked in the Flemish-Polish cross (where no animal in the F_2 generation exceeded the weight of the F_1 animals), but was much less marked in another cross, where the weight difference was not so great.

In 1922 Castle published an account of similar crosses which substantially confirmed the previous results of Punnett and Bailey. By crossing the Polish with the Flemish race, he obtained an intermediate F_1 generation; in an F_2 of 137 animals, the lightest animals came within the Polish range, but no rabbit appeared which approached in weight the two Flemish animals which constituted the Flemish stock for this experiment. Castle also agreed with Punnett and Bailey in finding that where the weight difference of the parent stocks is relatively small (as in his Polish-Himalayan cross) then in F_2 the heavier as well as the lighter parental classes are recovered.

Although there is in both of these experiments agreement as to the absence of the heavy animals in F_2 from the Polish-Flemish cross, both the experiments are open to the criticism that practically nothing was known about the variability of the parent stocks used, and that in any case only small numbers of F_2 animals were raised. Clearly the matter could not be left in this state of indecision: the issue which had been raised was of the utmost importance. If after taking every reasonable precaution, the heavier classes should still fail to reappear in the Polish-Flemish F_2, then that would in itself be a matter of fundamental genetic interest and of great practical consequence. If, however, in such an F_2 the complete range should be recovered, then it would be shown that the familiar Mendelian interpretation of multiple factors can be applied to weight inheritance in mammals in the same way as it has been applied to weight in birds.

Accordingly both the Polish and the Flemish stock used in the earlier Cambridge experiments were bred on with a view to getting data on the variability of the parent stocks (see Appendices), and in 1919 a cross was made between a Polish doe and a Flemish buck, the mating in 1912 having been in the reverse direction. As in the earlier experiments, the rabbits were kept in hutches in a wooden shed; there was no artificial heating, but the shed provided sufficient cover to mitigate the worst extremes of cold. The animals had an unlimited supply of hay, a liberal diet of bran and oats, and such fresh food (cabbages and roots) as the season provided. Apart from occasional victims of scour, the animals remained entirely free from disease, and no difficulty whatever was experienced in raising F_1 and F_2 rabbits to maturity.

While this experiment was in progress, Kopeć published an account of an experiment on the inheritance of birth weight in a Himalayan-Silver rabbit cross. The difference studied was small, the mean weight of the new born young in the two breeds being 36 and 44 gm. respectively, and there was considerable overlapping in the ranges of the two races. As Castle found in adult weights, so Kopeć found with birth weights, that where the difference between the parent stocks was small, the complete range could be recovered in F_2. While these results of Kopeć are of great interest, they have no direct bearing on the problem which forms the subject of this paper. It is very unlikely that the same factors which determine birth weight are also alone responsible for determining adult weight; and in any case the Flemish-Polish weight difference (being more than 2 to 1) is not to be compared with the mere 20 per cent. difference in Kopeć's Himalayan-Silver cross. Had Kopeć worked with

a wider weight difference, comparable to that of our Polish-Flemish cross, his birth weight records might well have constituted an interesting complement to our adult weight experiments.

It may be stated at once that in the present experiment, in an F_2 generation of 309 animals, the complete range of weights has been obtained, extending from the mean of the pure Polish stock at one end, to considerably beyond the mean of the Flemish stock at the other end of the scale. One may therefore claim to have established a *prima facie* case for the view that the inheritance of adult body weight in rabbits can be explained in terms of the well-known multiple factor theory. The matter is of some importance, because the failure of previous experimenters to recover the complete range of weights in F_2 has given currency to the notion that the Mendelian theory cannot be applied to weight inheritance in mammals. Indeed this statement has found its way into at least one text-book and has been cited (generally in a much more dogmatic form) in recent biological controversy.

With this brief statement of the principal result, we may turn to a more careful review of the experimental records and of the many unsolved problems to which this investigation has given rise.

2. THE POLISH-FLEMISH CROSS.

A general view of the weights of the parent stocks, and of the F_1 and F_2 generations, is given by Fig. 1, p. 264. The F_1 generation, consisting of 8 ♂♂ and 8 ♀♀, was bred from one mating only, the Polish ♀ (Q 127, 41 oz.) by the Flemish ♂ (Q 73, 93 oz.). It will be seen that the F_1 distribution is relatively compact, having a variability of 6·3 per cent. as against 10·0 per cent. for both the Flemish and the Polish stocks. The mean weight of the F_1 animals (69·9 ± 4·4 oz.) is just half way between the weights of the actual parent rabbits used; it is however considerably nearer to the mean of the smaller Polish stock than to that of the larger Flemish stock, being in fact exactly the harmonic mean of the two stocks. Clearly we have here no sign of hybrid vigour (at least as far as weight is concerned), and this notwithstanding that both our pure stocks had been closely inbred before crossing. Our results are in this respect in marked contrast to those of Castle and of Macdowell, both of whom obtained F_1 generations whose mean weights were nearer to the heavier Flemish stocks. This both Castle and Macdowell confidently regarded as being due to hybrid vigour, an opinion which gained support from the fact that the means of their F_2 generations regressed towards the lighter Polish stock. In view of a common tendency to regard hybrid vigour as

an invariable occurrence when two inbred strains are crossed, it is worth while noticing that in this Polish-Flemish cross we have an exception to the usual rule.

The F_2 generation consists of 309 animals bred from 8 does and 5 bucks more or less indiscriminately, 24 out of the possible 40 different matings having been made. Fig. 1 shows that the distribution is roughly unimodal
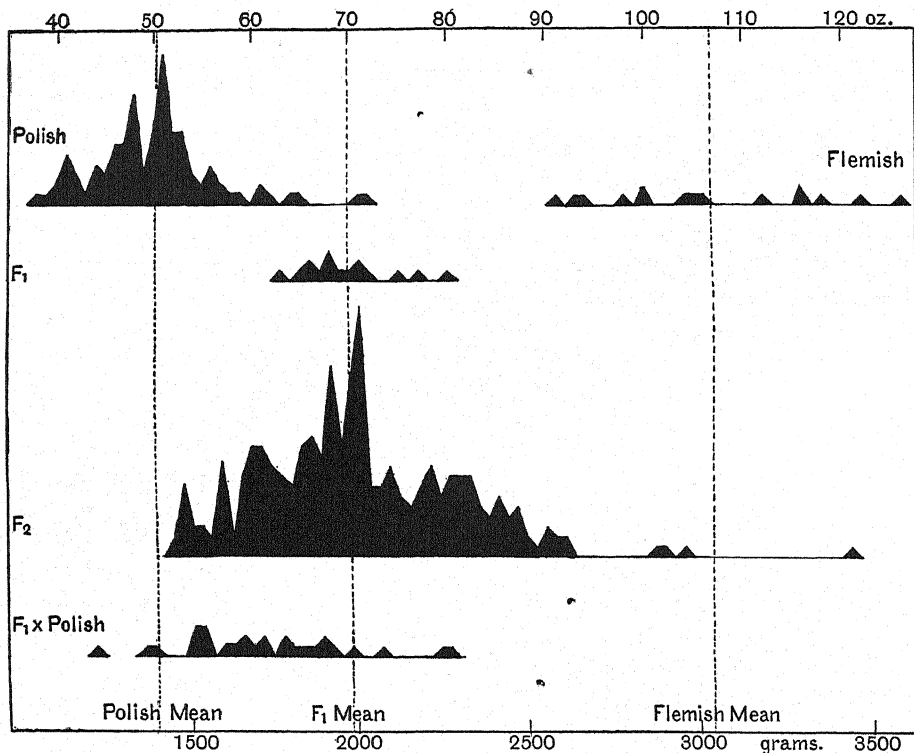

Fig. 1. The Polish-Flemish Cross.

and that the mean ($71 \cdot 2 \pm 10 \cdot 0$ oz.) is practically the same as that of the F_1 generation ($69 \cdot 9 \pm 4 \cdot 4$ oz.). It may be noticed that there is a faint suggestion of a second mode at about 60 oz. and of a third at about 80 oz., but the numbers are so small that it is not possible to determine whether these subsidiary modes are significant or merely fortuitous. Undoubtedly the most interesting point about this F_2 distribution is the fact that in it animals have been recovered as heavy as the Flemish grandparents. In fact eight F_2 animals come within the Flemish range, and of these three fall at about the mean of the pure Flemish stock, and one

(φ R 197) at 121 oz. is nearly as heavy as the heaviest Flemish animal bred in this experiment, namely φ Q 172, which weighed 126 oz. On the face of it, therefore, we have extracted in our F_2 generation animals as heavy as the original Flemish grandparent.

The minus end of the F_2 distribution falls well within the Polish range. It ends somewhat abruptly at 52 oz., just short of the Polish
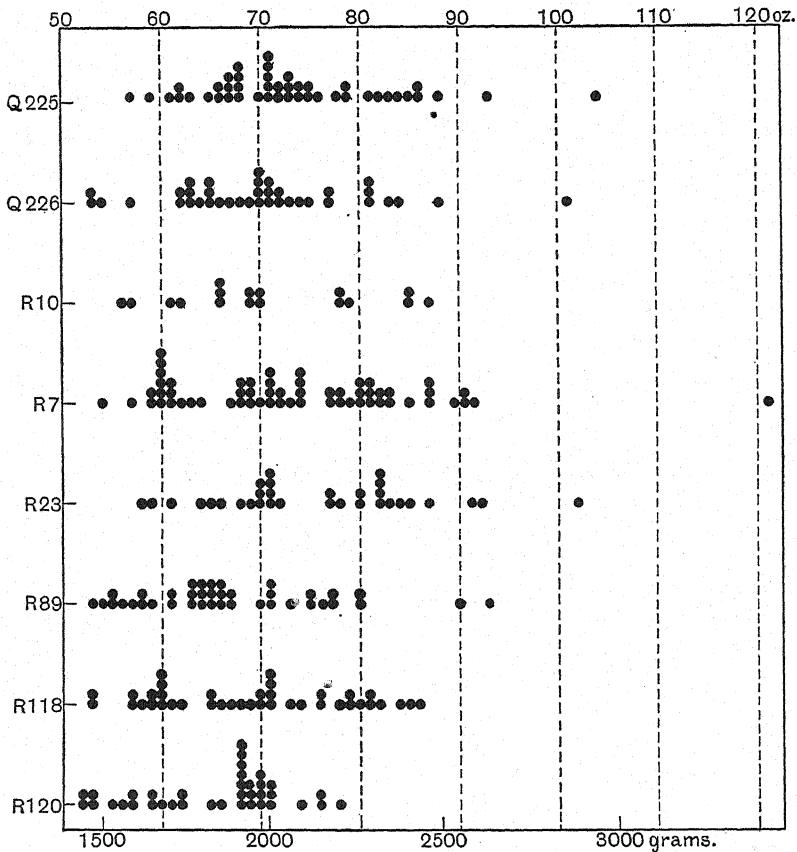

Fig. 2. To show the contribution of each $F_1 \varphi$ to the total F_2.

mean 50·2 oz., and several points short of the mean of the pure Polish descendants of φ Q 127 only (47·6 oz.).

Four F_3 families were bred from six of the lighter F_2 animals (see Fig. 6, p. 270) and although only 22 F_3 animals were raised, six of these fell below the mean of the Polish stock, and the others clustered closely together just on the positive side of the Polish mean. There is therefore

no doubt that, even if the F_2 range did not quite reach to the mean of the Polish stock, at any rate in F_3 we have obtained animals as light as the original Polish stock from which our cross was made.

Several interesting points emerge from a more detailed examination of the F_2 population. Fig. 2 shows the contribution of each F_1 doe to the total F_2. Clearly ♀ R 120 differs from the other does in that she bore no large animals, her heaviest offspring weighing 78 oz. (two) thus remaining within the range of the F_1 animals; the mean weight of ♀ R 120's family
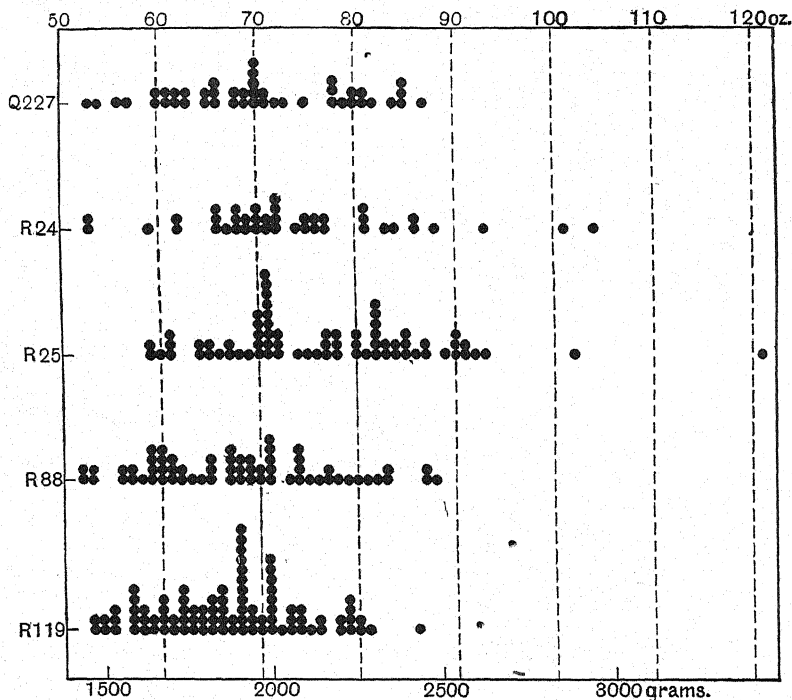

Fig. 3. To show the contribution of each F_1 ♂ to the total F_2.

is 64·9 oz. as against 71·2 oz. for the whole F_2 population. The variability of the whole F_2 is 14·1 per cent., but of ♀ R 120's family it is only 10·0 per cent., *i.e.* exactly the same as that of the pure Polish and Flemish stocks. By comparison with her sisters, we may conclude that she lacks one or more of the "heavy" factors which the other F_1 does contain. She was mated six times to ♂ R 88 and twice each to ♂ R 24 and to ♂ R 119. It is important to notice that she has in fact behaved just as did the F_1 animals in Punnett and Bailey's original experiment. The other 7 F_1 does seem to contribute about equally to the total F_2; R 118

has a slightly restricted range by comparison, but the difference is too small to be significant.

Turning now to the corresponding table for the F_1 ♂♂ (Fig. 3, p. 266), it is clear that ♂ R 119 gives a lighter family than do either ♂ Q 227 or ♂ R 88. But by far the most interesting thing is that all the heavy animals—the 12 over 90 oz. in weight—have been fathered either by
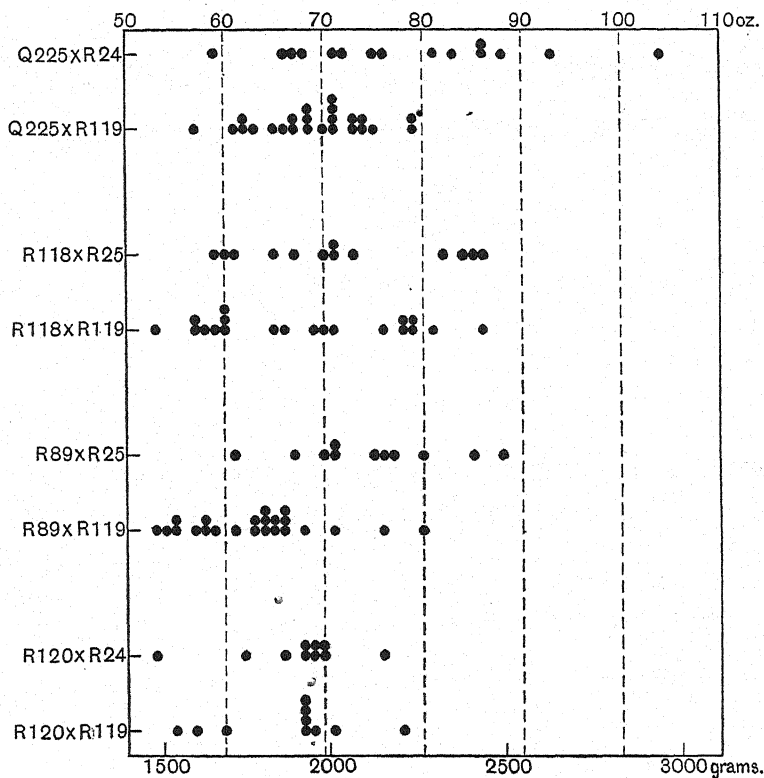

Fig. 4.

♂ R 24 or by ♂ R 25. If these two animals had by chance not been used, our F_2 distribution on this occasion would have been essentially similar to that found by Punnett and Bailey in 1918.

The contrasted genetic constitutions of ♂ R 119 on the one hand and of ♂ R 24 and ♂ R 25 on the other, are clearly shown (cf. Fig. 4) by comparing the family of Q 225 × R 24 with that of Q 225 × R 119; of R 118 × R 25 with that of R 118 × R 119; and of R 89 × R 25 with that of R 89 × R 119. Oddly enough where we might have expected

the sharpest contrast, *i.e.* in the case of ♀ R 120, there is none to be noticed; but the numbers are very small.

It is interesting to note that ♂ R 24 and ♂ R 25, notwithstanding their heavy offspring, are themselves almost the lightest of the F_1 animals, weighing at maturity (see p. 290) only 65 and 66 oz. each, while ♂ R 119 on the other hand was rather heavier, viz. 69 oz., just the mean of the F_1 distribution. The behaviour of ♂ R 24 and ♂ R 25 suggests that there is at least one factor which determines the growth of the very heavy animals, and that ♂ R 24 and ♂ R 25 contain this factor in contrast to ♂ Q 227, ♂ R 88 and ♂ R 119, who lack this factor. Since ♂ R 24 and ♂ R 25 are themselves not unduly heavy animals, we may conclude that this "heavy" factor is strictly recessive in its action. It would seem then, that in spite of continued inbreeding there is still considerable genetic impurity in our stocks, though whether in the Polish or in the Flemish, or whether indeed in both, it is not possible to say. One lesson however emerges from this analysis, namely, that an F_2 population should be raised not from one, but from many F_1 matings. It is more than likely that Punnett and Bailey's unexpected result was due to the fact that their Polish-Flemish F_2 was bred from only two matings amongst a family of F_1 animals which, as they themselves suggest, was almost certainly not homogeneous in its genetic make-up.

Turning again to Fig. 3, p. 266, one cannot help being struck by the discontinuity in the F_2 distributions, especially in the families of Q 227, R 25, and R 119. Q 227's family seems to fall clearly into two groups, one ranging from 53 to 73 oz. with a mean about 68 oz., and the other ranging from 78 to 87 oz. with a mean at 81 oz. Similarly R 25's family falls into two almost equal groups, the first ranging from 59 to 72 oz. with a mode at 71 oz., and the second ranging from 74 to 121 oz. with a mode at 82 oz. The discontinuity in R 119's family is less sharply marked, but there seems to be a large group ranging up to 75 oz. with a mode at 68 oz., and a smaller group ranging from 75 to 81 oz. with a mode at 79 oz. In the case of ♂♂ R 24 and R 88, the distributions are not incompatible with a break somewhere between 75 and 80 oz. in each case, but more than that it would be rash to claim.

It will be noticed that the families of both ♂ R 88 and ♂ R 119 show distinct clustering at the small end of the scale, in the former case with a mode at 60 oz. and in the latter with a mode at 57 oz. It is possible that this is not fortuitous; but large numbers would be required for a definite decision. This discontinuity at the light end of the scale is obviously less clearly marked than that already noted at the heavy end.

If these breaks are significant and are genetical in origin, an examination of the separate families of the F_1 ♀♀ should show similar discontinuous distributions. This they clearly do (Fig. 2, p. 265), especially in the cases of ♀♀ R 7, R 10, R 23, and R 118. Moreover, the families of ♀ R 7 and of ♀ R 118 show a trimodal distribution, similar to that of the family of ♂ R 119 already discussed above.

Further, an examination of the separate matings which make up the total F_2 should give still clearer evidence of discontinuity. Unfortunately, in order to prove a negative, large numbers are required, and the process
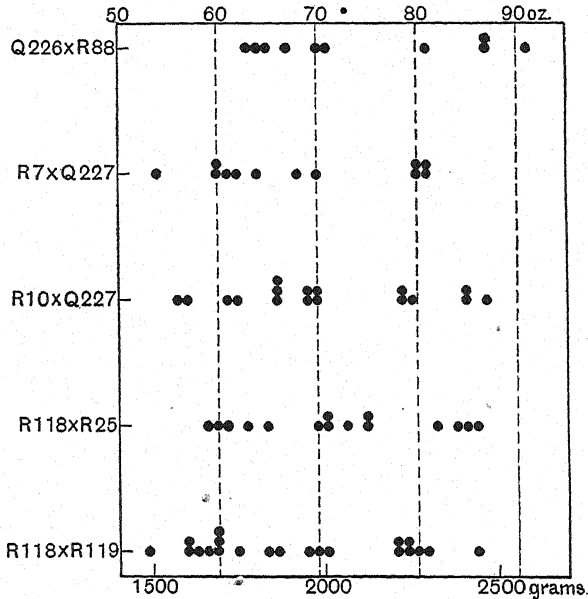

Fig. 5. To show discontinuous distribution in some F_2 families.

of breaking up the F_2 into its constituent families necessarily reduces the numbers in each case. However of the 23 families which make up the F_2, 19 consist of two or more litters each, and of these 19 families there are five (set out in Fig. 5, above) which show marked discontinuity. Owing to these breaks not being coincident, the process of summation blurs the gap, and in the F_2 frequency curve we get no more than the faint indication of a break, to which attention has already been drawn.

Taken all together, the evidence for discontinuity is not sufficiently strong to demonstrate the action of one predominating weight factor.

On the other hand, one would hesitate to say that the F_2 distribution is indubitably unimodal, especially after a study of the constituent families. This is disappointing, for it leaves undecided one of the most interesting points in connection with weight inheritance, viz. whether we are dealing with the effect of a single predominant factor, or with the
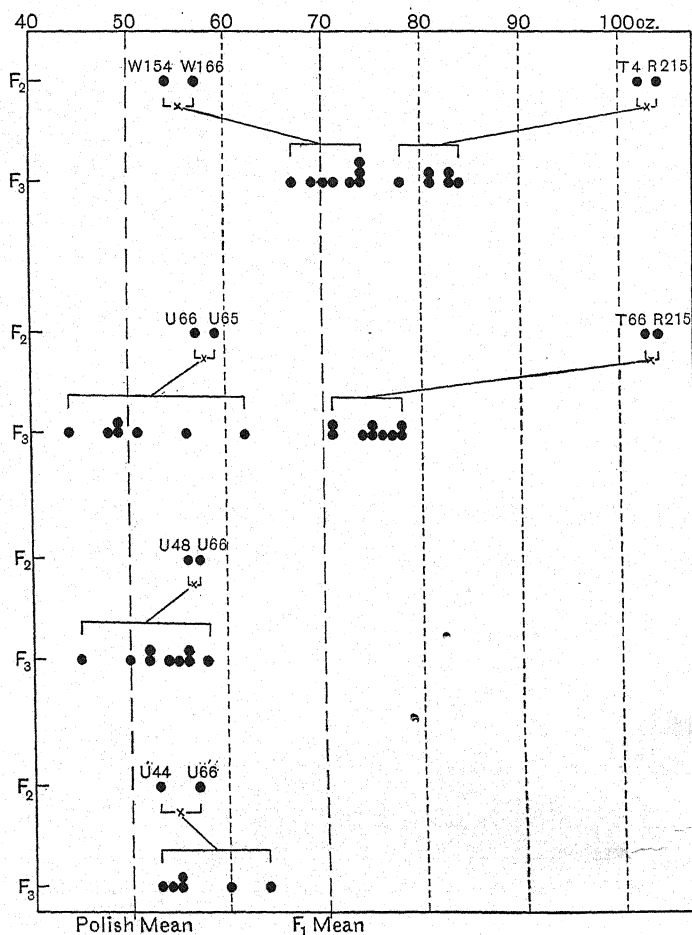

Fig. 6. Some F_3 families.

combined effect of several nearly equally potent major factors. The experimental records are too few to decide whether we should regard the F_2 distribution as a $1 : 2 : 1$ distribution, blurred by the action of many modifying factors, or whether the distribution is really unimodal

and therefore due to several equally strong weight factors. It is quite possible that the appearance of trimodality in the F_2 frequency curve is due to a chance piling up of the 68, 70, and 71 oz. classes.

A small back-cross generation (Polish $\times$ F_1) has been bred, whose weight distribution is shown in Fig. 1, p. 264. As far as it goes, there is no indication of discontinuity (such as would be expected if there were but one predominating weight factor); but of course a sample of 28 animals is far too small to throw decisive light on the matter.

In any case, the greatest possible caution must be exercised in making any positive deduction from the form of an F_2 frequency curve. By constructing a number of frequency curves for 1, 2, and 4 factors and assigning different values to the standard deviations of the biotypes involved, Tedin has shown convincingly how easy it is to fit to a given frequency distribution a Mendelian theory based on 1, 2, or 4 factors[1]. In view of this, I prefer to regard the question of the number of weight factors involved in this experiment as undecided.

It has already been noted that some F_2 animals have been extracted which have bred true for light weight. The distributions of these F_3 families, which are shown in Fig. 6, p. 270, are in accordance with expectation and call for no special comment. On the other hand, all attempts to select a pure breeding heavy family from the heavy F_2 animals ended in failure. The one heavy F_2 buck (R 215, 104 oz.) was mated to ♀ T 4 (102 oz.) and to ♀ T 66 (103 oz.) and in both cases gave F_3 families fairly compact in distribution, but one averaged just over, and the other considerably under, 80 oz. (see Fig. 6, p. 270). The one really heavy F_2 doe, R 197, which weighed 121 oz., gave with ♂ R 215 a far heavier F_3 than did either ♀ T 4 or ♀ T 66; but here again the average of the F_3 family (90 oz.) was much below the weight of either of the parents, and only one animal (T 224, 120 oz.) exceeded 100 oz. (Fig. 7, p. 272).

The heaviest F_3 buck, T 225 (99 oz.), was mated to the F_3 ♀ T 224, and the resulting F_4 family showed a further regression towards the F_2 mean, the average weight being 84 oz. Seven F_5 animals have so far been raised in this line from the heaviest F_4 animals, and again we get a further move towards the small end of the scale, as is clearly shown by Fig. 7, p. 272.

Two other F_3 pairs were selected, a fairly heavy couple (♂ U 154, 96 oz., and ♀ U 155, 99 oz.) and a lighter couple (♂ W 60, 79 oz. and

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[1] Unpublished, but quoted in full (with curves and figures) by Rasmusson, *Hereditas*, x, pp. 117–24.
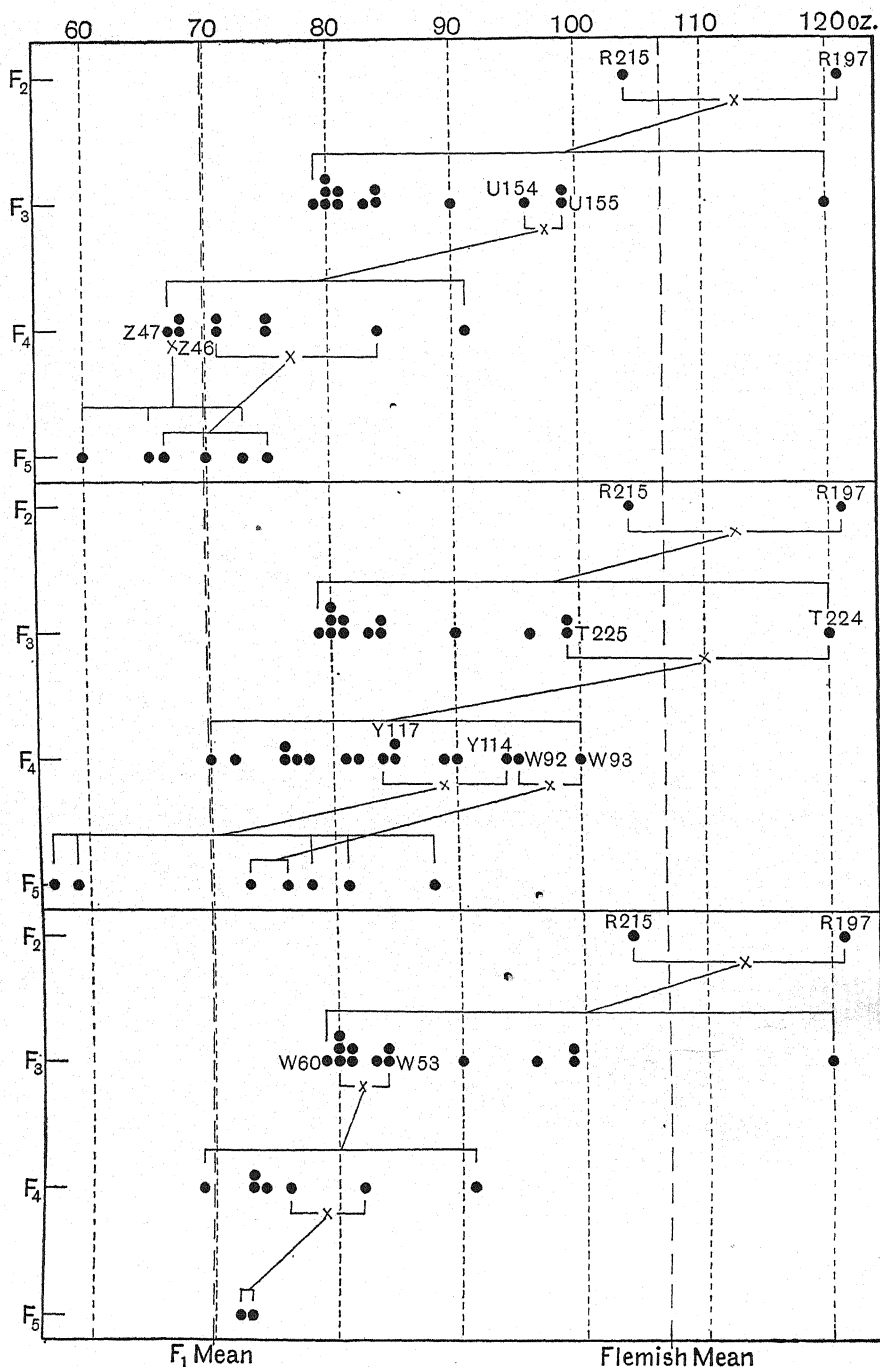
Fig. 7. To show the diminishing weight in successive generations of rabbits bred from heavy F_2 animals.

♀ W 53, 84 oz.). In spite of the wide difference in the weights of these two F_3 pairs, their F_4 families were almost the same in average weight; if there is anything to choose, the F_4 from the heavier pair was on the average the lighter of the two (see Fig. 7, p. 272). In both cases F_5 showed a further move towards the light end of the scale.

A plausible explanation of this on ordinary Mendelian lines does not seem possible. Neither R 215 nor R 197 admit of any doubt as to their weights (see p. 290). Even if we dismiss T 4 and T 66 as obvious heterozygotes, the same can scarcely be said of R 197, which should clearly contain the full complement of weight factors. Yet she gave out of a family of 14 only a single young one weighing over 100 oz.; and in F_4 no animal weighing over 100 oz. was obtained, though the heaviest F_3 animals were used as parents.

It might be argued that R 215 and R 197 were exceptionally favourably nourished animals. R 215 was one of four in a litter of which two died within five days of birth: it is true then that R 215 may have had an undue share of milk. The same may be said of R 197. But even if we grant this to be the explanation of the extreme weight of R 215 and R 197, there remain other cases to be explained. T 224 was one of three; U 154 and U 155 were a litter of two, but they are lighter, not heavier than T 224 and T 225. And if small litter size were the determining factor, it is difficult to understand why Z 46 and Z 47, also a litter of two, should be the lightest of all the "heavy" animals so far bred.

The steady move towards the light end of the scale is far too regular and consistent in every case and in each generation to warrant an explanation of chance good nurture. Fig. 7, p. 272, bears a striking resemblance to Fig. 12 on p. 20 of Punnett and Bailey's original paper, and we are to-day as much at a loss for an explanation of this phenomenon as were those authors ten years ago.

Some of the lightest of these F_5 "heavy" animals have been mated to the Polish animals in the hope that a comparison of the weight distribution of the progeny of this mating with the original F_1 distribution may throw some light on what genetic change, if any, has taken place in the course of inbreeding these extracted heavy F_2 rabbits.

An interesting picture of how the weight factors act can be obtained by transposing the frequency curves given in Fig. 1 on to the one-way logarithmic scale. This has been done in Fig. 8, p. 274, and it is at once clear that the data so arranged present an almost perfectly symmetrical appearance. The Flemish range which looks so ragged in Fig. 1, p. 264, is now seen to be quite as compact as the Polish distribution. The F_1

and F_2 means now fall just half way between the Polish and the Flemish means, and the F_2 distribution is practically symmetrical about its mode. The four F_2 stragglers over 100 oz.—so very noticeable in the ordinary frequency curve on p. 264, still lie some way beyond the main body of the F_2 animals, but they have now been brought in much closer towards

Logarithm of weight.
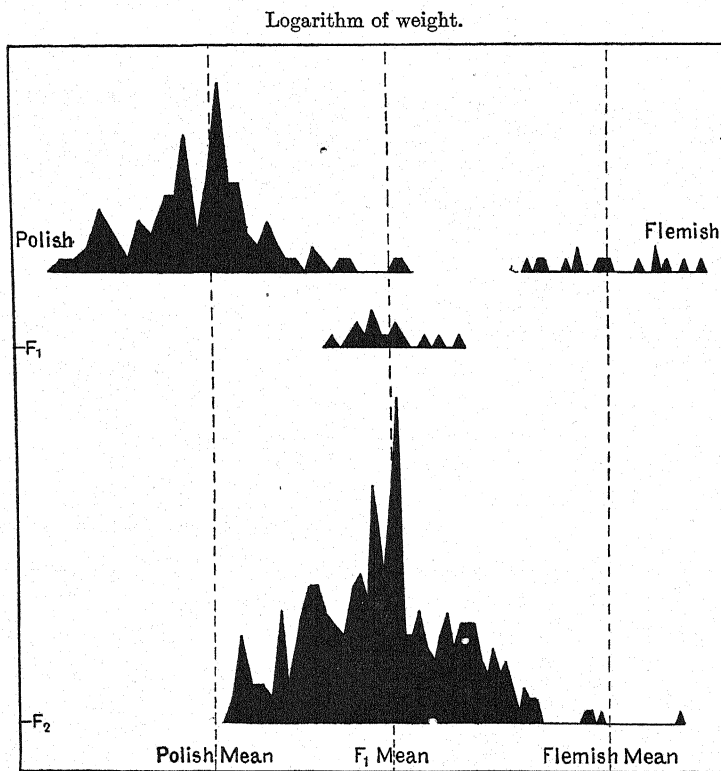
Fig. 8. The Weight Distribution on one-way Logarithmic scale.

the mean. The conclusion to be drawn from this diagram is that weight factors act in a strictly logarithmic manner, as has indeed been assumed in previous factorial analysis of weight inheritance, *e.g.* in Punnett and Bailey's well-known case in poultry.

SUMMARY AND CONCLUSIONS.

This, then, for the time being brings to an end the account of our Mendelian analysis of weight in the Polish-Flemish rabbit cross. We have found:

(1) That the F_1 is intermediate in weight and shows no sign of hybrid vigour.

(2) That in F_2 the entire range of weights is obtained, but that this entire range is only given by the progeny from a few of the F_1 animals. Most of the F_1 animals give only a restricted F_2 range, such as both Punnett and Bailey, and Castle, found in previous experiments.

(3) That the numbers are insufficient to determine whether or not there is a single predominating weight factor.

(4) That some of the light F_2 animals bred true in F_3 for light weight.

(5) That it was impossible to find a pair of heavy F_2 animals which bred true for heavy weight.

(6) That by arranging the weight frequency curves on a logarithmic scale it becomes at once clear that weight factors act in a logarithmic manner.

3. APPENDICES.

I. *The Polish Stock.*

The Polish rabbits used in the present experiment are descended from the Polish buck used in Punnett and Bailey's original experiment. The animals have been under observation for nine generations, matings having been made as far as possible sister to brother, with a view to studying the effect of inbreeding on weight. At the same time records were kept on fecundity, and for this purpose nearly all the animals were tested by mating both "in" and "out"—latterly all have been so tested. Several of these inbred Polish animals turned out to be completely sterile; three (T 174, W 62 and W 163) were nearly but not quite absolutely sterile. By her 13th mating ♀ T 174 had one young which she promptly deserted; but though mated twice again she never bred any more. Similarly ♀ W 62 and ♀ W 163 both produced one young each by their 7th and 5th matings respectively; but though subsequently mated, neither ever produced young again, nor did either of them suckle their solitary offspring. There is therefore considerable ground for including these animals in the list of sterile animals given in Table I, p. 276. One interesting point about the sterility of these animals in contrast to the sterility in some of the Flemish does (see p. 286) is that while the latter were fertile by their first matings and then became totally sterile, the Polish does which we have been considering only bore at the ages of 25, 19, and 20 months respectively.

In the males in every case the immediate cause of sterility was apparently cryptorchy; but in the females there was, except in one

TABLE I.

Sterility in the Polish stock.

Generation	Sterile animals	Weight (oz.)	Total animals	Ratio of sterile : total
I	—	—	3	—
II	—	—	6	—
III	—	—	6	—
IV	♀ R 164 ♀ R 167 ♀ R 114 ♀ R 139	48 51 71 47	20	4 : 20
V	♂ T 233 ♀ T 174	50 72	21	2 : 21
VI	♂ Y 8 ♂ W 44 ♂ U 70	44 52 43	23	3 : 23
VII	♀ W 163 ♀ W 62	62 61	15	2 : 15
VIII	—	—	6	—
IX	—	—	5	—

The average weight of the sterile animals is 55·2 oz.

case, no obvious anatomical defect. The five completely sterile does were all carefully examined after death, and in four cases the ovaries appeared normal, with apparently healthy follicles and corpora lutea. It should be noted that similar cases of ovaries apparently normal histologically were found in the sterile Flemish rabbits, *e.g.* in Q 217 and Q 221 (p. 285). In the Polish doe R 139, however, the ovaries were distinctly small; they contained no corpora lutea and the follicles were recorded as unusually small and undeveloped. Apart from this case, the only respect in which the sterile Polish may be regarded as abnormal is that some of the animals were extraordinarily fat. It is well known that farmers usually regard very fat animals as shy breeders, so it is no matter for surprise to find in rabbits also that fatness and sterility go hand in hand—though which is cause and which effect it would be rash to say[1].

A comparison of the weights of the sterile and fertile Polish animals shows that the "stragglers" at the heavy end of the scale (so noticeable in Fig. 1, p. 264) are sterile. The average weight of all the sterile animals is 55·2 oz. against 49·6 oz. for the fertile stock. The question arises whether we ought not to exclude these sterile animals in arriving at the "average weight" of the Polish stock for comparison with the Flemish. If we leave them out, the average weight of the stock is only very slightly reduced (from 50·2 oz. to 49·6 oz.). But on the other hand,

[1] Parkes and Drummond (*Brit. Journ. Exp. Path.*, 1928, ix, p. 63) incline to the view that in rats fatness is an effect rather than a cause of sterility.
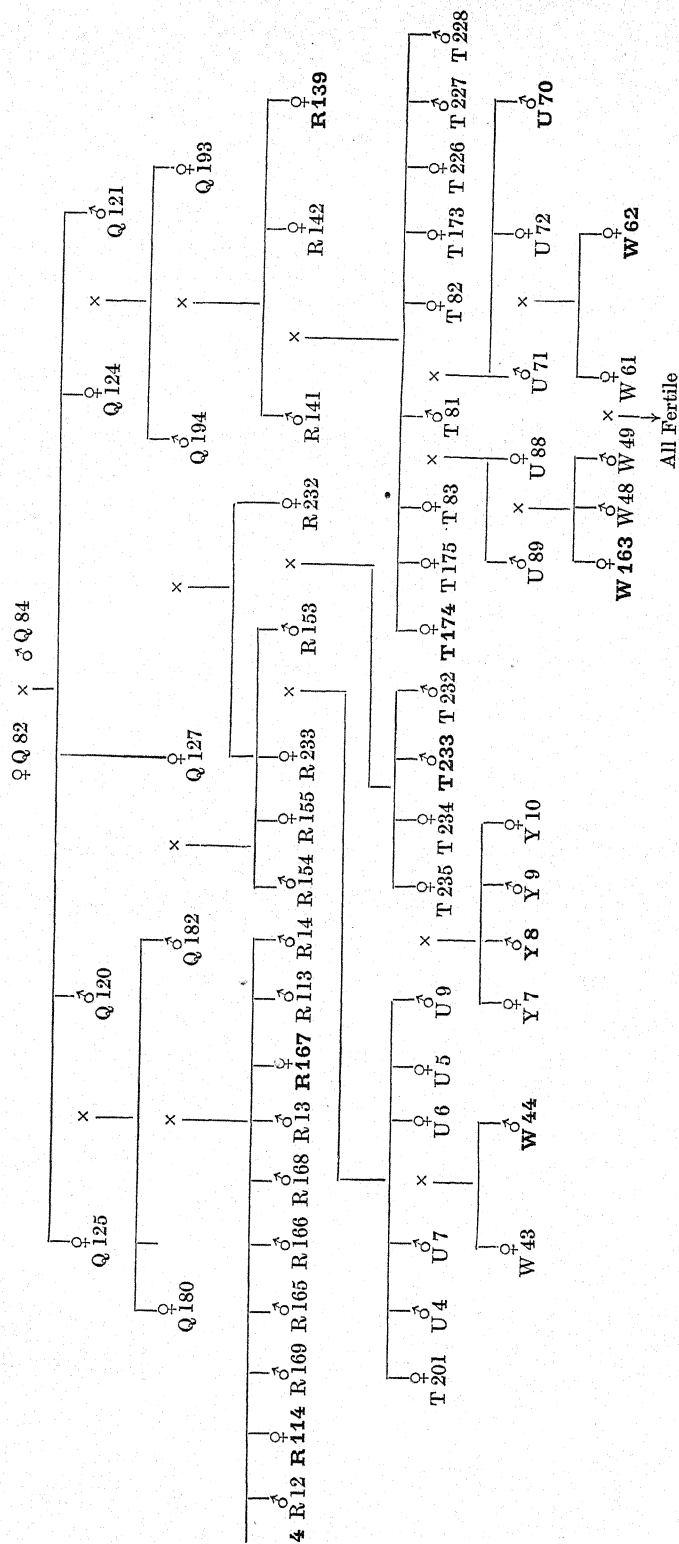
PEDIGREE OF STERILE POLISH RABBITS

Fig. 9. Sterile animals are shown in heavier type.

owing to the exclusion of the "stragglers," we get a much more compact distribution, which has a variability of 8·7 per cent. as against 10·0 per cent. for the whole stock. At first sight, therefore, there would seem to be good ground for leaving out these sterile animals. But against this must be set the fact that nearly all the Flemish animals recorded in this experiment were sterile. Since these Flemish were also abnormally fat, we should, for the same reasons, have to exclude most of the Flemish stock from our consideration. On the whole we incline to the view that for purposes of comparison the simple straightforward plan of including all the animals, fertile and sterile, is probably the wiser course.

The incidence of sterility in the Polish stock is shown by the pedigree table given in Fig. 9, p. 277. It will be seen that the sterility occurs for the first time in the 4th generation, but disappears again after the 7th generation. And even in the 7th generation the two animals in question, W 62 and W 163, were, as we have seen (p. 275), not absolutely sterile, though they were nearly so. It is too soon to say definitely whether sterility has been bred out of this stock—time alone will provide the answer.

TABLE II.

Sterility in Polish rabbits.

Mating	Total young	Fertile	Sterile
Q 180 × Q 182	12	9	3
Q 193 × Q 194	3	2	1
R 142 × R 141	9	8	1
R 232 × R 153	4	3	1
T 82 × T 81	3	2	1
T 235 × U 9	4	3	1
U 6 × U 7	2	1	1
U 72 × U 71	2	1	1
U 88 × U 89	3	2	1
	42	31	11

The most interesting thing about the incidence of sterility in the Polish stock is brought out by putting together those families which contain one or more sterile animals. This has been done in Table II above and it can be seen at once that we have a very close 3 : 1 ratio for the fertile : sterile animals. Indeed if we exclude the slightly doubtful animals W 62 and W 163 and their sibs, we get exactly 27 : 9. Though the numbers are very small they give ground for suspecting the action of a single Mendelian factor. How this factor acts we cannot say. This much however is clear; if the sterility in this stock is due to a single factor, then this factor affects both sexes equally, at least as far as the end result of sterility is concerned.

We may now turn to a consideration of the effect of inbreeding on other characters of the Polish stock. Fig. 10 below shows the effect on weight and on litter size in successive generations. Inbreeding has clearly produced no decline in the weight of the animals—a marked contrast to what was found in the Flemish stock (p. 289). As regards litter size, there is a clear falling off between the 4th and the 9th generations, though the average litter size in the 2nd and 3rd generations is not appreciably greater than that in the later generations. Where non-Polish males were used, the average size of the litters was usually greater than in the cases where Polish fathers were used. The two exceptional generations (the
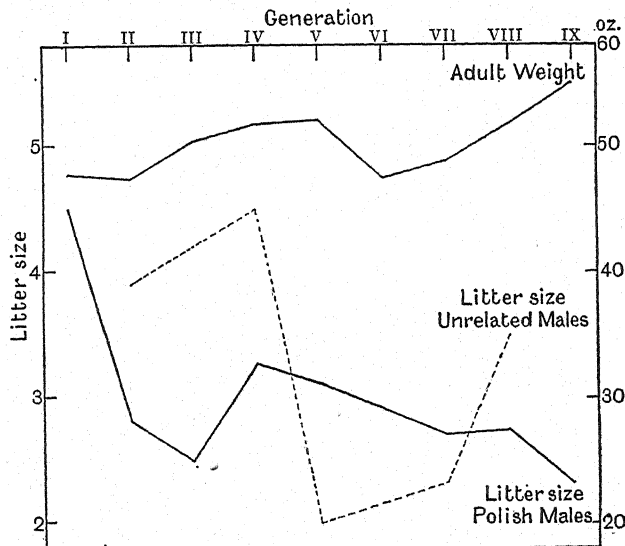
Fig. 10. The effect of inbreeding on Weight and on Litter size in Polish rabbits.

5th and the 7th) were not really fair cases. For here the only Polish does put to males of other breeds were those which had failed to breed with their own kin—i.e. they were the most sterile of the fertile does. In the other generations, where there was no selection of does, the cross-bred litters were always larger than the pure Polish litters.

Throughout this experiment there has been considerable difficulty in getting the Polish does to rear their young, though the stock was reputed originally to be one of good mothers. The proportion of litters in each generation totally abandoned at birth is shown in Fig. 11, p. 280. In the case of the pure bred stock, this proportion (which we have called

Fig. 11. Effect of inbreeding on the Desertion Quotient in Polish rabbits.
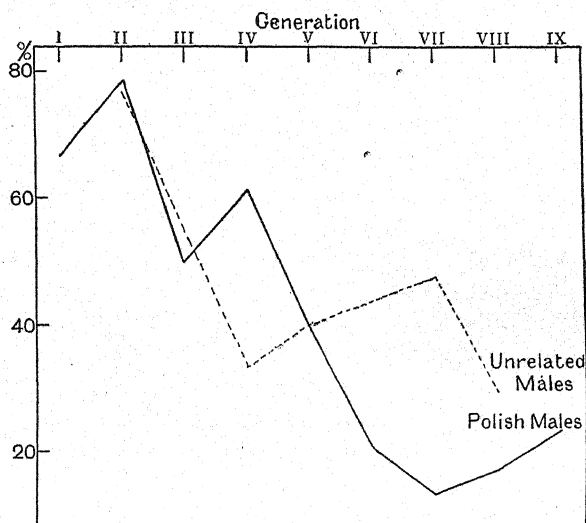
Fig. 12. Effect of inbreeding on Viability in Polish rabbits.

the "Desertion Quotient") steadily rises, reaching nearly 80 per cent. in the 7th generation and then falling somewhat in later generations. It is interesting to note that in the cross-bred litters, the desertion quotient is much less in the 7th and 8th generations; this probably means that the cross-bred young are more sturdy and more persistent suckers, and thus succeed in stimulating a reluctant flow of milk into activity.
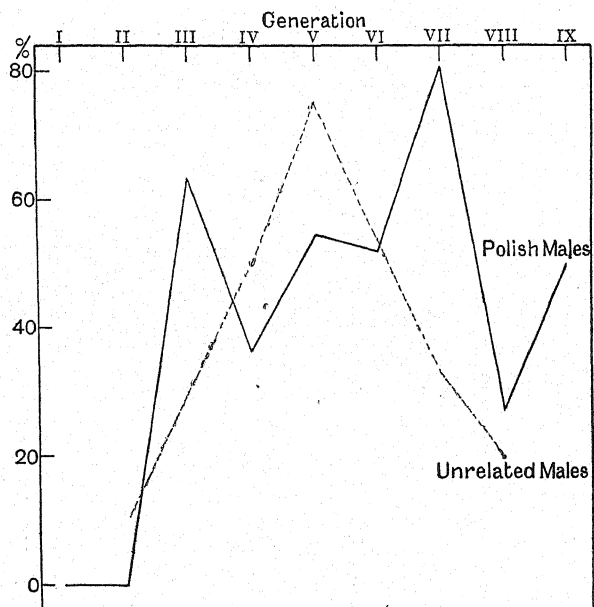
Nearly related to the desertion quotient is the "Viability" of the stock, as measured by the proportion of young born which are actually
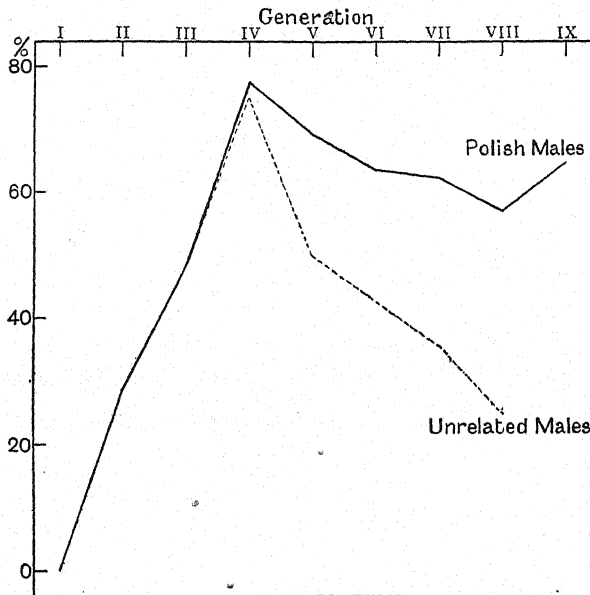
Fig. 13. Effect of inbreeding on the percentage of infertile matings in Polish rabbits.

reared to maturity by their own mothers. Here there seems to be, as we should expect, a steady drop in the viability of the pure Polish from nearly 80 per cent. in the 2nd generation to 10 per cent. in the 7th generation. Again it is clear that the viability of the cross-bred stock shows no significant falling off between the 4th and the 8th generation, thus pointing to a more vigorous constitution for the cross-bred than for the pure-bred animals (Fig. 12).

Another measure of sterility is the percentage of infertile matings. Fig. 13 above shows that this index rises very rapidly from nothing in the 1st generation to a maximum of nearly 80 per cent. in the 4th

generation; it then gradually declines to 60 per cent. in the 8th generation, but rises again sharply in the 9th generation. The corresponding curve for the non-Polish males closely follows that of the pure-bred stock up to the 4th generation, but then falls away rapidly in later generations, showing that the later Polish does, though very infertile with Polish bucks, are quite fertile with males of other breeds. The very high proportion of sterile matings in the 4th generation is, of course, due to the four completely sterile ♀♀ which turned up in this generation and which were repeatedly mated in order to test their sterility.

TABLE III.

Statistical Summary of Sterility in Polish Rabbits.

Generation	Number of mature animals	Average weight (oz.)	Polish males					Unrelated males				
			Number of litters	Average size of litters	Desertion quotient %	Viability %	Percentage of sterile matings	Number of litters	Average size of litters	Desertion quotient %	Viability %	Percentage of sterile matings
I	3	47·7	2	4·50	0	66·7	$\frac{0}{2}=0$	—	—	—	—	—
II	6	47·4	5	2·80	0	78·6	$\frac{2}{7}=28\cdot5$	10	3·9	10·0	77·1	$\frac{4}{14}=28\cdot5$
III	6	50·4	13	2·50	61·5	50·0	$\frac{12}{25}=48\cdot0$	—	—	—	—	—
IV	20	51·7	11	3·27	36·4	61·4	$\frac{38}{49}=77\cdot5$	2	4·5	50·0	33·3	$\frac{6}{8}=75\cdot0$
V	21	52·1	19	3·12	55·5	39·6	$\frac{45}{64}=69\cdot3$	4	2·0	75·0	40·0	$\frac{4}{8}=50\cdot0$
VI	23	47·5	25	2·91	52·0	20·2	$\frac{43}{63}=63\cdot5$	—	—	—	—	—
VII	15	48·8	27	2·71	80·5	13·8	$\frac{44}{71}=62\cdot1$	9	2·3	33·0	47·6	$\frac{5}{14}=35\cdot8$
VIII	6	51·8	12	2·75	27·2	17·1	$\frac{19}{28}=57\cdot1$	6	3·5	20·0	28·7	$\frac{2}{8}=25\cdot0$
IX	5	55·0	6	2·33	50·0	23·3	$\frac{11}{17}=64\cdot7$	1	5·0	—	—	—

The numbers in these experiments are too small to warrant comparisons with Sewell Wright's classic experiments on inbreeding, but bearing in mind Wright's conclusions we may summarise our own results in the Polish stock as follows:

(1) It would seem that absolute sterility has been bred out of the stock at the 6th or 7th generation.

(2) On the average the sterile animals are markedly heavier than the fertile animals.

(3) A consideration of the families in which the sterile animals arose points to the working of a single recessive sterility factor.

(4) There has been a rapid diminution in the fertility of the pure stock as measured by (*a*) the desertion quotient, (*b*) the viability, and (*c*) the proportion of sterile matings—in the last case the sterility seems

to reach a maximum in the 4th generation, while in the first two cases the decline in fertility is more or less continuous.

(5) There is evidence to show that the cross-bred young in each generation have not suffered in constitutional vigour from the inbred condition of their parents.

(6) The litter size shows some decline, but it is only a very small and very gradual one.

(7) The weight of the animals raised to maturity shows no decline whatever.

On the whole, we may conclude that whereas the sifting out of a single recessive factor may well account for the incidence of absolute sterility, the diminishing fertility, as shown by the various indices used, is very difficult to account for on any such simple hypothesis.

II. *The Flemish Stock.*

The pedigree of the Flemish stock is shown in Fig. 14, p. 284, which includes and extends that given on p. 20 of Punnett and Bailey's original paper. It will be noticed that all the animals have descended from a single pair, almost entirely by sister to brother mating. At the 5th generation further progress came to an abrupt end, owing to the sudden appearance of total sterility. It is true that in the 4th generation one of the does, ♀ Q 72, persistently deserted her young, and in the light of our present knowledge we realise that that should have been a significant warning of the oncome of sterility—but at the time little attention was paid to it and ♀ Q 72 was discarded as soon as young were obtained from her sister ♀ Q 74. But in the 5th generation the sterility was complete; neither I nor Mr Hammond (to whom at a later stage were handed over many of these sterile animals) succeeded in raising any more pure Flemish from this stock. An exhaustive account of the abnormal histology of these animals will be found in Chapters II and VII of Hammond and Marshall's *Reproduction in the Rabbit*, and only the points of genetic interest will be discussed here. In the first place, it should be said that, apart from their sterility, all the animals (except Q 223) were splendidly healthy; they were well covered with fat, their coats were fine and glossy, and to all outward appearance they were thoroughly creditable specimens of their breed. Inbreeding, therefore, so far from having caused any general debility or loss of vigour, seems rather to have had a strictly specific action on the reproductive vitality of these animals.

FLEMISH PEDIGREE.

♀ O 105 ✕ ♂ N 169

♂ O 135
♀ O 136
♂ O 137
♀ O 138 ✕
♂ O 139
♂ O 202 ✕
♂ O 201 ✕
♀ O 203
♂ O 204
♀ O 205

♂ P 76
♀ P 79
♂ P 80
♀ P 77
♂ P 61
♀ P 56
♀ P 57
P 58
♀ P 59
♀ P 60
♀ P 62

♂ P 78

♀P 228
♂ P 225
♀ P 226
♀ P 227

♀ Q 72
♂ Q 222
♂ Q 223
♀ Q 74 ✕
♂ Q 73
Q 172
Q 173
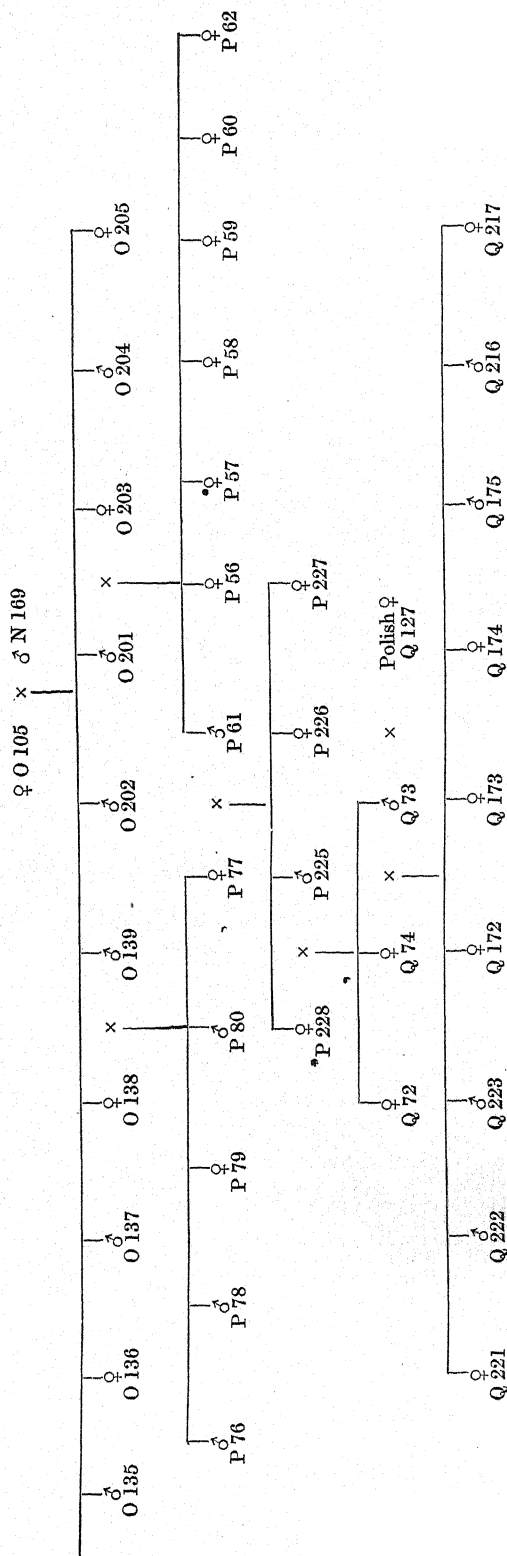Polish ♀
Q 127 ✕
♀ Q 174
♂ Q 175
♂ Q 216
♀ Q 217

♀ Q 221

Fig. 14.

Of the five sterile does, Q 172 never bore any young, though repeatedly mated, both to her brothers and to unrelated bucks. Both her ovaries were infantile and histologically abnormal[1].

Her sister, ♀ Q 173, when about a year old bore young twice to her brother ♂ Q 175, though she deserted both her litters at birth. Subsequently she was often mated, both to her brother and to unrelated bucks, but she only produced one litter of 2 at about two years old. At *post mortem* examination the ovary and corpora lutea appeared normal; the uterus was pregnant, but most of the foetuses were in a condition of atrophy.

Another sister, ♀ Q 174, at sixteen months old bore to her brother Q 175 one litter of 5, which she deserted at birth. Subsequently she produced 2 young by an unrelated buck and reared them successfully. This is in agreement with our observations in the Polish stock, where we found a greater viability in the cross-bred young than in the pure-bred. But though frequently mated after her second litter, she never again bore young. *Post mortem* examination showed the ovaries and corpora lutea to be normal; the uterus was pregnant, but contained several atrophic foetuses.

The fourth doe, Q 221, though often mated to her brothers Q 175 and Q 222, never bore them any young. By an unrelated buck, however, she had, at the age of 20 months, one litter of 2 young; but these were the only offspring to which she ever gave birth.

Finally Q 217, like Q 172, was completely sterile however mated. But in this case the ovaries and corpora lutea appeared normal at *post mortem*, and the uterus showed the usual changes of pseudo-pregnancy.

Arranged in series we may say that Q 172 was completely sterile with degenerate ovaries; Q 217 though sterile had apparently normal ovaries; it may be supposed that her ova were incapable of development. Q 221 was in her youth only very reluctantly fertile, and then only to an unrelated male. In later life she was completely sterile, but her ovaries and uterus (though not pregnant) appeared entirely normal at *post mortem*. Both Q 173 and Q 174 were in their youth fully fertile in so far as young were readily born: their "infertility" at this stage, it would seem, consisted in the inability of the pure-bred young to suck their mother, for we have seen that Q 174 successfully reared a litter of

[1] In this and in many of the following cases, the animals had been handed over to Mr Hammond, and the *post mortem* examinations were accordingly in these cases made by him; I am deeply indebted to him for permission to use here in abbreviated form the accounts published by him in full in his book on the rabbit already cited.

cross-bred young. In later life these two does failed to produce any live young at all.

It should be noted that the three litters of pure Flemish obtained from ♀ Q 174 and ♀ Q 173 contained 5, 5, and 3 young each, numbers small compared to the 7 and 8 obtained in the 1st generation, but bigger than the litters obtained in the 3rd and 4th generations. At *post mortem* these does had 7 and 5 foetuses, in each case 3 being recorded as normal. But in view of the repeated failure of these two rabbits to give birth to any live young at all, we may conclude that these apparently "normal" foetuses would in time also have become atrophic. The interesting point is that in the first two litters of ♀ Q 173 and of ♀ Q 174 most, if not all, of the fertilised ova appear to have developed to full term and young were born alive; whereas in later life no pure-bred young whatever were produced, though the Flemish male used, ♂ Q 175, was known at that time to be fertile with unrelated does.

Many geneticists regard the presence of atrophic foetuses as the experimental proof of the action of lethal factors, but in this case if lethal factors were the immediate cause of sterility, it is not easy to see why they should not have worked their fatal effect in the first two litters of Q 173 and Q 174. Unless we are to make the very improbable assumption that a lethal mutation arose somewhere in the germinal tract of each of these two does, it is difficult to avoid the conclusion that the cause of sterility is to be sought rather in some physiological condition which quickly supervened, and at an unusually early stage in the life of these does rendered them incapable of carrying their young to full term. Lethal factors there may well have been, but presumably they were present in these animals during their whole lifetime. It is possible that in the first full flush of youth the does' reproductive systems may have been active enough to overcome the action of the lethal factors, and that the smallest falling off in the does' reproductive vitality allowed the lethal factors to work their fatal effect. But this explanation assumes as a necessary subsidiary hypothesis a normal dropping off of reproductive vigour at the ages of 13 and 16 months respectively, and moreover, as happened in these cases, during the spring and early summer, a time when the breeding season is usually regarded as being at its height. There is, as a matter of fact, no evidence that rabbits normally show any sign of decreasing fertility till at least four years old.

Of the bucks, ♂ Q 223 was early recorded as a poor rabbit and was discarded without any attempt having been made to breed from him.

His brother ♂ Q 216 was outwardly normal; he copulated several times with his sister ♀ Q 217 without producing any young. Owing to lack of hutch space he was killed at a relatively early age; no *post mortem* examination was made, interest in sterility not having been aroused at this stage of the experiment. We were thus left with ♂ Q 175 and ♂ Q 222, the former having already had two litters by his sisters ♀ Q 173 and ♀ Q 174. Subsequently he failed to give any young when mated to his sisters and only rarely did he have young by unrelated does: in later life he became entirely sterile, the testes being withdrawn permanently into the body cavity and showing degenerative histological changes. The remaining buck, ♂ Q 222, turned out to be completely sterile. At *post mortem* the testes were found to be abdominal, though it appeared that at one time they had been scrotal. Histologically they were very degenerate.

Thus the sterility in the Flemish males, as in the Polish males, appears in every case to be associated with cryptorchy. It is interesting to note that ♂ Q 175, like his sisters ♀ Q 173 and ♀ Q 174, was fully fertile in his first breeding season; but subsequently he became sterile though at an abnormally early age. We have living to-day (1928) two of the original Polish-Flemish F_1 bucks now eight years old; though they show many outward signs of old age, they are still fully fertile[1].

There is one very serious difficulty about explaining the sterility of this Flemish stock as a simple sifting out of one or more lethal factors. In the ordinary way, a lethal factor acts as in Cuénot's mice and in the well-known case of Kerry-Dexter cattle by killing a proportion of the foetuses. It is difficult to see how an ordinary Mendelian factor can, from the nature of our mating, bring about the atrophy of *all* the foetuses. There may be a lethal factor which contributes to the end result of total sterility, but in that case it would be incorrect to describe such a factor as being the cause of the total sterility in this stock.

There remains the suggestion that there may be at work a sterility factor as such, which produces sterility in both sexes in a manner at present unexplained. The Polish stock afforded some evidence for assuming the existence of some such simple Mendelian factor. But clearly an equally simple hypothesis for the Flemish stock meets with difficulties. In the first place, all the pure Flemish descendants of ♂ Q 73 and many of the pure Polish descendants of ♀ Q 127 were sterile. Hence on the sterility factor hypothesis, ♂ Q 73 certainly, and ♀ Q 127

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[1] One of these has since died, apparently of old age; but he remained fertile almost to the end.

probably, carried one or more of the supposed sterility factors. Even if the same factors had not been brought in from both sides in the Polish-Flemish cross, it is curious that although over 500 descendants of the mating ♀ Q 127 × ♂ Q 73 have been reared and many have been bred from, no case of sterility has so far come to light, either in F_2 or in any subsequent generation, at any rate up to F_6. It might, of course, be argued that the sterility in the pure stocks was not due to a single factor *per se*, but to a complex constellation of factors which after long in-
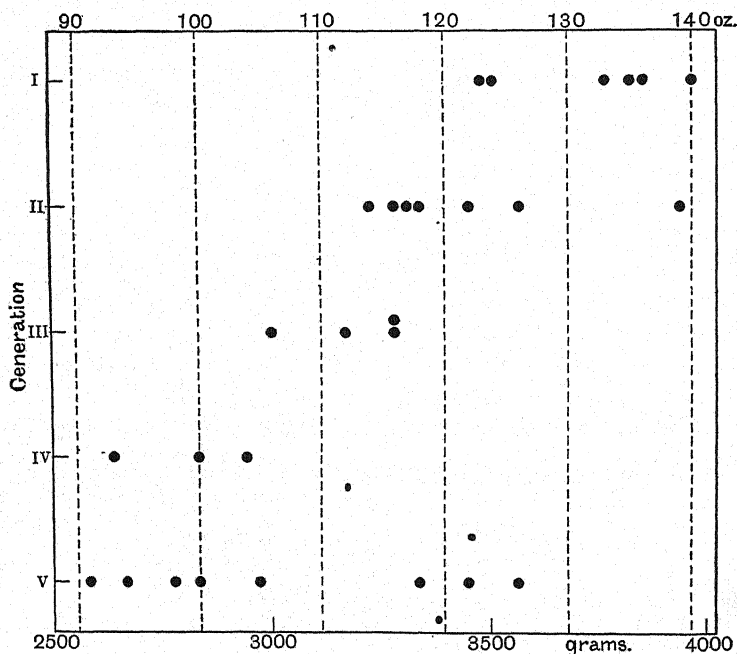
Fig. 15. To show the decline in Weight of the Pure Flemish rabbits in successive generations.

breeding (we may fairly safely assume that both the Polish and the Flemish stock had been long inbred by the Fancy before coming into our possession) had become nearly homozygous in those of the Flemish stock which we had by chance selected to breed from. Such a constellation of factors would be broken up by crossing and might well not be re-formed by random assortment in subsequent generations. But by introducing some such subsidiary hypothesis, the attractive simplicity presented by the notion of a sifting out of a recessive factor is shattered, and it is doubtful if the sterility factor hypothesis thus modified gets

us much further in forming a picture of the process by which the sterility in these animals may have been brought about.

A much more serious difficulty about any sterility factor hypothesis—at least in its simple form—arises from the incidence of the sterility in the Flemish stock. All the animals in the final 5th generation were the offspring of ♀ Q 74 × ♂ Q 73, both these animals being normally fertile. If the sterility were due to a sifting out of a recessive factor, then both ♀ Q 74 and ♂ Q 73, though themselves fully fertile, must have been heterozygous for the supposed sterility factor. It is very unlikely that, where expectation is 3 fertile to 1 sterile, we should have got two litters of 4 and 5 young all of which (with the possible exception of ♂ Q 223 who was not tested, see p. 286) turned out to be sterile.

It must, therefore, be confessed that while we find it difficult to reconcile the foregoing considerations with the notion of a simple sifting out of one or more recessive sterility factors, we find it equally difficult to advance any other hypothesis which would provide a connected account of the peculiar incidence of sterility in this Flemish stock.

In Punnett and Bailey's original paper, attention was drawn to the effect of inbreeding on weight. Fig. 15 (which includes that given on p. 20 of Punnett and Bailey's paper) shows the diminishing weight of the Flemish stock from generation to generation; the decrease goes on as far as the 4th generation, but in the 5th there is a sharp rise. Analogy with the Polish stock suggests that this increase in weight is associated with the sterility in this generation. It is perhaps worth noticing that ♀ Q 72, whose case was discussed on p. 283, was the heaviest of the animals in the 4th generation.

In view of this inexplicable decrease in weight, it is not easy to decide how much of the Flemish material should be taken into account when calculating the average weight of the Flemish stock for comparison with the Polish stock. The buck ♂ Q 73, used as the heavy parent for the experiment, was one of the lightest Flemish bred, weighing only 93 oz. at maturity. He was one of the three animals which constituted the 4th generation (see pedigree, Fig. 14, p. 284): all things considered it seems the most reasonable course to include only the animals of the preceding and succeeding generations in order to obtain a fair sample of the Flemish stock. This gives us 15 animals with a mean weight of 106·7 oz. and a variability of 10·0 per cent. As this happens to be exactly the variability found for the Polish stock, it would not be unfair to conclude that both stocks have reached about the same degree of purity as regards weight factors.

III. *The Growth Curve.*

The form of the growth curve of rabbits was discussed at length in Punnett and Bailey's paper, to which reference has already been made, and to this discussion the present experiments have very little new to add. A typical growth curve of one of our F_2 rabbits is shown in Fig. 16.

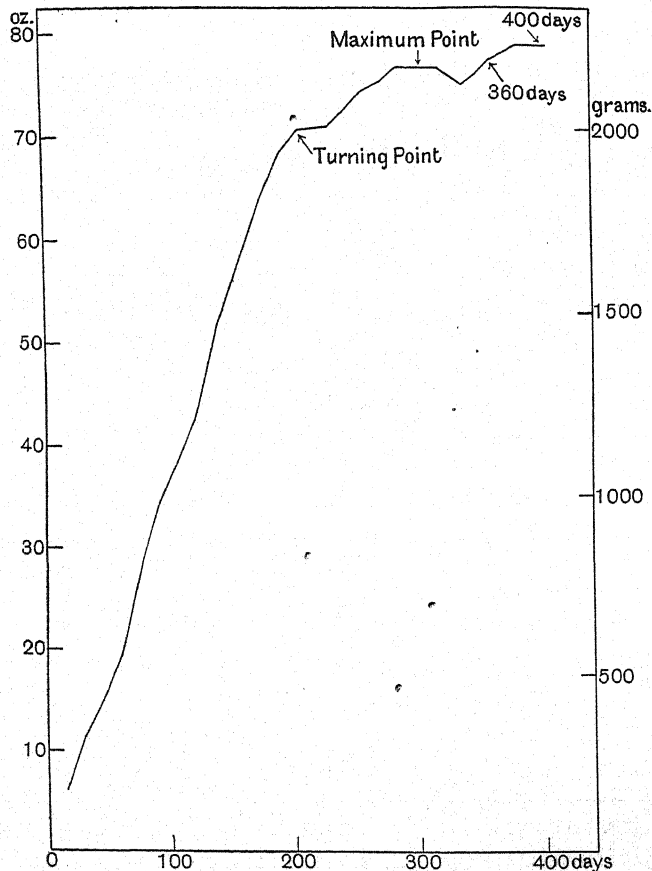Fig. 16. The growth curve of a rabbit ($\male$ U 210, Polish-Flemish F_2).

It will be seen that at first the graph shows a rapid and almost uniform rise. At some point, generally between 180 and 250 days, the rate of growth slackens off, usually quite abruptly, and so much so that it sometimes even becomes negative; the point where the curve thus turns sharply we have called the "Turning Point." The graph gradually rises

from the Turning Point to a first maximum (which we have called the "Maximum Point"), then declines again slowly, finally rising once more till adult weight is reached at about 400 to 500 days.

In previous experiments with rabbits, "the weight of a rabbit" has usually been taken as the greatest weight attained by the animal under the age of one year. This has the great advantage of being an entirely objective measure; but it suffers from the serious drawback of being an arbitrary measure, which does not necessarily bear any relation to the rate of growth of the animal. Two rather more natural points—natural in the sense that they might well bear a definite relation to some physiological change—would seem to be the Turning Point and the Maximum Point as defined above. The Maximum Point is the less clearly marked of the two, and moreover the rabbit has to be kept alive for a long time, in order that a clear conspectus of the whole growth curve may be obtained. And, even so, it is often then found that there are several Maximum Points, usually near together as far as weight is concerned, but more or less widely separated in point of time. The "Maximum Point" is therefore distinctly unsatisfactory as a measure of weight, in that it cannot by any means always be related to the quantity, "number of days required to reach the Maximum Point."

On the other hand, the Turning Point is usually (though not always) clearly defined, both as to weight and as to time; it comes relatively early in the rabbit's life, so that the hutches can generally be cleared under 300 days, a matter of great practical importance to the experimenter. Table IV shows the extent to which the Turning Point was

TABLE IV.

	Turning Point sharp	Turning Point not sharp, but determinable	Turning Point altogether indistinct
Flemish	14	1	—
Polish	75	23	3
F_1	14	1	1
F_2	273	36	2

clearly marked in the growth curves of the animals under observation. As might be expected, the Polish curves with their gentle gradients give a higher proportion of indistinct Turning Points.

In order to make a comparison, the weight distributions of the animals have been set out in Fig. 17, p. 292, with respect to the two "natural" points (the Maximum Point and the Turning Point) and with respect to two arbitrary measures, namely the greatest weight attained under 360 days and under 400 days respectively. As a matter of fact it
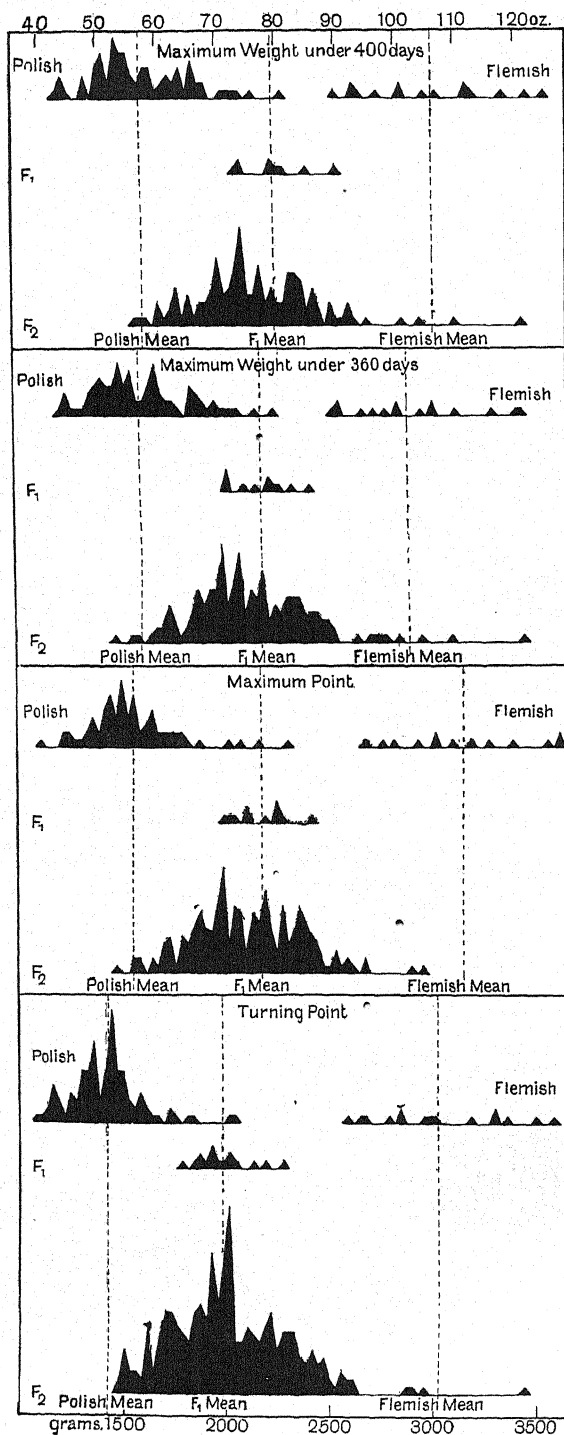
Fig. 17. Different methods of measuring the Weight of a rabbit.

will be seen that the same type of frequency curve is obtained by each system of classification, nor would there seem to be anything to choose between the four methods so far as standard deviations are concerned (see Table V below). Each system has a different set of means; but the distributions of types about these means as measured by the coefficients of variability are almost identical.

TABLE V.

To show the Number of animals (N), *the Mean weight* (M), *the Standard Deviation* (σ), *and the Variability* (V), *for the four different ways of measuring the weight of rabbits.*   •

	Polish				Flemish			
	N	M (oz.)	σ (oz.)	V %	N	M (oz.)	σ (oz.)	V %
Turning Point	101	50·2	5·0	10·0	15	106·7	10·7	10·0
Maximum Point	78	54·7	7·3	13·6	13	110·7	10·9	9·8
Max. wt under 360 days	85	56·8	7·8	13·7	15	102·1	10·2	10·0
Max. wt under 400 days	81	57·6	7·8	13·6	15	106·1	10·5	10·0

	F_1				F_2			
	N	M (oz.)	σ (oz.)	v %	N	M (oz.)	σ (oz.)	v %
Turning Point	15	69·9	4·4	6·3	309	71·2	10·0	14·1
Maximum Point	11	76·4	3·9	5·1	191	74·1	8·9	12·1
Max. wt under 360 days	11	76·8	4·5	5·9	182	75·1	9·8	13·0
Max. wt under 400 days	9	79·4	5·2	6·6	142	76·5	10·0	13·2

All things considered, therefore, I have used the "Turning Point" as defining the weight of a rabbit for the purposes of this experiment, for it offers the practical advantage of allowing the hutches to be cleared early, and the theoretical advantage of bearing a definite relation to growth of the animal.

It would clearly be of interest to know what physiological significance (if any) can be attached to the Turning Point. Ostwald's observations on mice (cited by D'Arcy Thomson) would suggest that this retardation of growth may be related to the oncome of puberty. In order to test this suggestion for rabbits, pairs of brother and sister rabbits from the same litters were left together in the same hutches, weighed at regular intervals, and the dates of the first litters noted. Under these conditions it was found that the ♀ usually became pregnant as soon as she was capable of bearing young and in consequence her Turning Point vanished —the increased weight due to pregnancy more than counterbalancing the normal retardation of growth at this period. Now if the Turning Point coincides with the oncome of puberty in both sexes, the following types of case should be found in these experiments with such ♂ and ♀ pairs.

Type A. Here the ♂ shows an ordinary Turning Point, but the ♀ shows none, owing to the ♂ having reached puberty at the same time as the ♀. In this type, then, a litter should be born about 31 days after the Turning Point of the ♂. But as our rabbits are only weighed every fortnight, the Turning Point as shown on the growth curve is only a rough approximation—there may be an error of 15 days one way or the other. There have been included in Type *A*, therefore, all cases in which the age of the ♀ at the birth of her first litter *minus* the age of the ♂ at his Turning Point falls within 16 and 46 days. It will be seen that there are ten such cases set out in Section A of Table VI below.

TABLE VI.

The Relation of the Turning Point to Puberty.

Pair	I Turning Point of ♀ (days)	II Turning Point of ♂ (days)	III Age of ♀ at birth of first litter (days)	Column III less Column II	Column III less Column I	Reference to text
U 177 × U 176	—	210	256	46	—	
Z 20 × Z 18	—	200	226	26	—	
Z 34 × Z 35	—	170	186	16	—	
Z 95 × Z 97	—	222	244	22	—	
A 9 × A 7	—	180	206	26	—	
A 51 × A 52	—	217	257	40	—	A
A 89 × A 90	—	213	248	35	—	
A 156 × A 155	—	180	200	20	—	
A 236 × A 238	—	258	286	28	—	
C 6 × C 9	—	257	294	37	—	
Y 227 × Y 226	175	203	234	31	—	B
Y 230 × Y 232	220	249	276	27	—	
W 191 × W 190	—	160	210	50	—	
Z 186 × Z 188	—	150	210	60	—	
Z 187 × Z 185	—	170	222	52	—	
A 17 × A 14	—	167	220	53	—	C
A 88 × A 87	—	170	232	62	—	
C 7 × C 8	—	257	322	65	—	
C 28 × C 29	—	190	246	56	—	
C 180 × C 179	—	150	215	65	—	
U 180 × U 181	284	284	358	74	74	D
Y 43 × Y 44	238	238	352	114	114	
U 162 × U 161	252	252	154	− 98	− 98	E
A 112 × A 113	—	283	217	− 66	—	
W 201 × A 199	—	141	174	33	—	F
W 201 × C 137	—	188	206	18	—	

Type B. This class comprises the two cases in which the ♀ appears to have reached maturity before the ♂. Here we find the ♀ Turning Point comes first, followed by the ♂ Turning Point, the litters arriving 31 and 27 days respectively after the ♂ Turning Point. It is perhaps worth

noting that on the whole we find it uncommon for the ♀ to reach puberty in advance of the ♂.

Type C. This group may be said to be the converse of Type *B*, consisting as it does of cases in which the ♂ matures considerably before the ♀. Here the interval between the ♂ Turning Point and the arrival of the first litter is greater than 46 days. Table VI, p. 294, shows eight such cases in Section C.

Type D. There are two cases (Section D of Table VI) which fit the theory only if the further assumption is made that the ♀ rabbits may attain puberty without necessarily conceiving immediately. For in these two cases both ♂ and ♀ Turning Points are clearly marked and the litter was in each case born 74 and 114 days after the Turning Points, both ♂ and ♀. We have noticed that cryptorchids, though infertile, show normal Turning Points at about the usual age; in one of the cases under consideration, *descensus testiculorum* was observed to be delayed and almost certainly accounted for the late arrival of the first litter. In the other case no observation was made on this point, so that delayed *descensus* may well have been the cause here too. It is also just possible that miscarriage may have occurred unnoticed.

Type E. Finally there are two cases (Section E of Table VI) which cannot be made to fit the theory that the usual retardation at the Turning Point is due to puberty. In the case of U 162 × U 161, sharply marked Turning Points in ♂ and ♀ are shown at 252 days—but a litter had already been born at 154 days. This is an astonishingly early litter and means that both ♂ and ♀ must have reached sexual maturity at 4 months old. This is quite an exceptional case, and it may be that the Turning Point is an indication of puberty only within a certain range of time. In the second case the ♀ (A 112) had young at 217 days, but the father (A 113) did not show his Turning Point till he was 283 days old, nearly 100 days after the conception of his first litter. It is true that his curve shows a slight irregularity at 150 days; but it is very slight in comparison to the obvious Turning Point at 283 days and in the ordinary way it would have excited no attention at all. It must be confessed that it is difficult to see how to bring these two cases into line with the others which have so far been discussed.

Another obvious test of the notion that the Turning Point is an indication of the oncome of puberty—in the buck at any rate—is to allow one ♂ out of a litter to grow up with his mother and note the relation of his Turning Point to the time at which his mother bears him a litter. This has been done in two cases (Table VI, Section F) and it will

be noticed that the young were born 18 and 33 days respectively after the Turning Points of the bucks used. As far as they go then, these two cases afford confirmation of the suggestion here put forward.

On the whole the evidence goes to support the idea that the Turning Point represents the oncome of puberty, though the difficulty presented by the two exceptional cases described above should not be lost sight of. However it is clear that the method of fortnightly weighings is far too rough a measure to settle the matter. Daily weighings and a much more elaborate regulation of the environmental factors would be necessary in order to test the hypothesis thoroughly.

IV. *The Relation of Weight to Sex, Colour, and Litter size.*

We may now turn to consider certain factors which may perhaps affect the weight of rabbits. In the first place there is sex. Punnett and Bailey found that the ♀♀ were nearly always heavier than the corresponding ♂♂. They had no data for the Polish, and in their Flemish stock the difference was not noticeable; but in their F_1 and F_2 animals from the Polish-Flemish cross there was a marked difference in favour of the does.

TABLE VII.

To show the Number of animals (N), *the Mean weight* (M), *and the Standard Deviation* (σ), *for the male and female rabbits in the Polish-Flemish cross.*

	Polish			Flemish			F_1			F_2		
	N	M (oz.)	σ (oz.)	N	M (oz.)	σ (oz.)	N	M (oz.)	σ (oz.)	N	M (oz.)	σ (oz.)
Males	48	48·7	4·4	5	99·5	5·4	8	70·5	5·1	149	70·9	9·5
Females	53	51·3	7·20	10	109·5	10·7	7	68·9	3·1	159	71·5	10·1

The records of the present experiment have been arranged to test this point, and as Table VII above shows, what slight difference there is on the average in favour of the ♀♀ is insignificant when judged by the ordinary statistical rule. One odd thing may however be noticed, that the ♀♀ are nearly always more variable than the ♂♂, markedly so in the pure Polish and Flemish strains, but much less so in the F_2. If it should turn out to be a general rule that for weight the ♀ is the more variable sex in rabbits, then that would enable the conclusion we arrived at above (that sex has no effect on average weight) to be reconciled with the fact that the show standards for several important breeds prescribe a heavier weight for the doe than for the buck.

In a very interesting investigation into size inheritance in the tomato, Lindstrom has shown that there is genetic association between colour and weight, thus pointing to linkage between well established colour factors and the hypothetical size factors. A similar sort of linkage between size and pattern factors in *Phaseolus* has been shown to exist

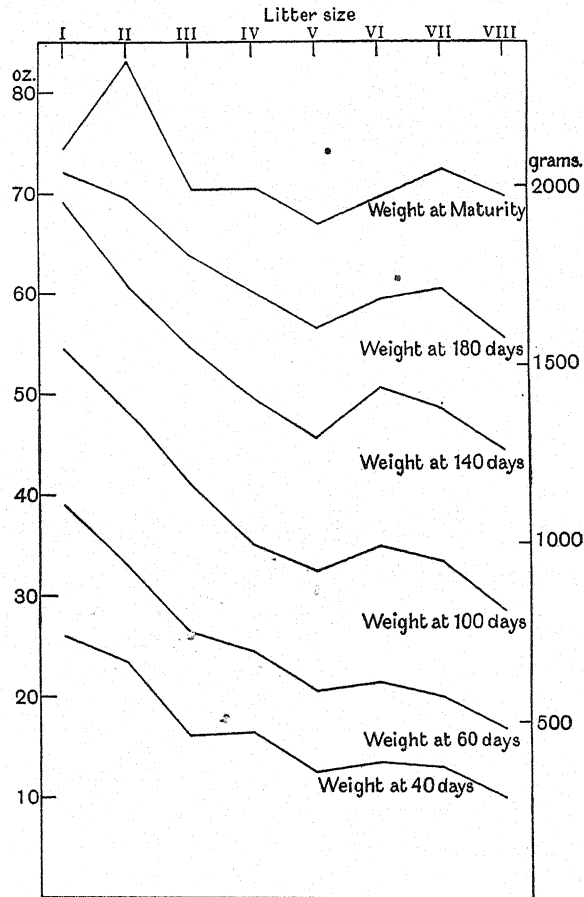Fig. 18. Effect of Litter size on the growth of F_2 rabbits.

by Sax and by Sirks, and quite recently another such case of linkage between size and colour factors has been described by myself in the Kohl Rabi. The records in the rabbit experiment, however, yield no evidence whatever for any such linkage between weight factors and the well-known factors which differentiate the common fur colours, or the

recently discovered factor which determines the fat-colour in rabbits.
The data on the relation of pattern to weight are too scanty to warrant
any conclusion.

Punnett and Bailey agree with Macdowell in finding that litter size
has no effect on the adult weight of rabbits. On the whole the present
data bear out this view; but, as Fig. 18, p. 297, shows, it would seem that
the superior start which litters of one and two are able to get, produces
some slight effect on the weight at the Turning Point. The average
maturity weight of the rabbits from the ten litters of one and two is
81·2 oz. as against just 70 oz. for the remaining F_2 population and 69·3 oz.
for the three litters of 8 young each. But the scatter in the weights of
the rabbits from the smaller litters is large—the standard deviation being
no less than 16 oz.—so that the difference between 81 oz. and 70 oz. is
scarcely significant.
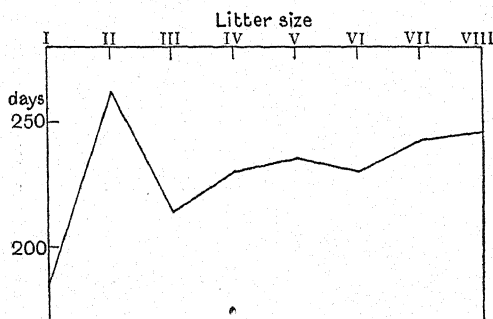
Fig. 19. The effect of Litter size on maturity time in F_2 rabbits.

Though the effect of litter size on adult weight is only doubtfully
significant, its effect on growth is indubitable, as the curves in Fig. 18,
p. 297, show. The litters were usually weaned at about six weeks old, and
therefore, as would be expected, the relative effect of litter size on weight
is much greater at this stage than at any later period in the rabbit's life,
the single animals being at 40 days old $2\frac{1}{2}$ times as heavy as those from
litters of 8.

The question naturally suggests itself whether the retarding effect
of large litters on growth may not persist, so that the rabbits from
large litters take longer to reach maturity than do the rabbits from
smaller litters. The records on this point, as Fig. 19 above shows, are
inconclusive. While there is obviously a gradual lengthening of the
period required to reach maturity as we pass from litters of 3 to those
of 8, yet the rabbits from the litters of 2 are the slowest of all to reach

the Turning Point, their average "Maturity Time" being 260 days as against 246 days for litters of 8.

V. *On the Time taken to reach Maturity.*

The choice of the Turning Point as the measure of weight led naturally to an interest in the rate of maturing as measured by the number of days taken by the rabbit to reach the Turning Point, a quantity which we may call the Maturity Time. Unfortunately the interest and importance of this aspect were not fully realised until after the experiment had been launched; for this reason, it was for weight only that the stocks were purified and the original parents selected. A glance at Fig. 20, p. 300, shows that both the Polish and the Flemish stocks are mongrel for maturity time. With respect to this character the Polish rabbits range from 120 days to 390 days with a mean at 235 days, but without any obvious mode. The variability is 21 per cent., more than twice that of the same stock in respect of weight. Even if we take only the descendants of ♀ Q 127, we get almost as big a range, quite as big a variability, and an equally obvious absence of a mode. It is therefore very unlikely that ♀ Q 127 was anything but mongrel for maturity time. It should be noticed that ♀ Q 127 was herself relatively slow in maturing, her Turning Point being at 280 days, as against 235 days for the average of the Polish.

Fig. 20 also shows that the Flemish stock is no purer than the Polish, its range being from 210 days to 460 days. On the whole it is clearly a more slowly maturing strain than the Polish. Its mean is 383 days with a variability of 18 per cent., and as in the Polish case, there is no obvious mode. Except for one rabbit, all the Flemish animals matured at over 300 days. This one animal which matured at 210 days, clearly quite exceptional in this respect, was unfortunately the one to be chosen as the original Flemish buck for crossing with the Polish doe. We are not able to judge how far his exceptionally rapid growth was "accidental" or in any way genetic in origin.

Thus with regard to maturity time, we are in the unfortunate position of having made a cross between impure stocks, and with the actual parents used not even average of their kind. With this inauspicious beginning, it is not surprising that the Polish-Flemish weight material as it stands gives no clear light on the problem of maturity time as an inheritable character. Fig. 20, p. 300, shows the Polish-Flemish material arranged with respect to maturity time. It will be noticed that the F_1 distribution is compact (though small in number) and shows a clear mode at 210 days, the mean being 227 days. Punnett and Bailey found that

their F_1 animals matured much more rapidly than the parent strains. In the case of our F_1 rabbits, though their average maturity time of 227 days is significantly shorter than that of the Flemish stock (283 days), it is clearly not appreciably shorter than the average maturity time of the Polish stock (235 days) or of the F_2 population (232 days). Neither in
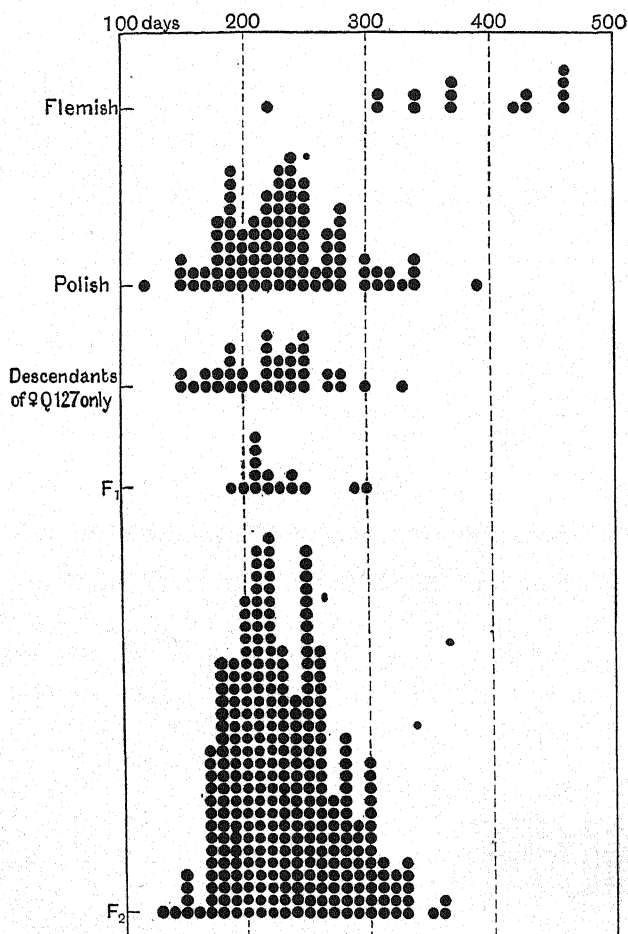
Fig. 20. Maturity time: weight distribution.

weight nor in maturity time, therefore, does there seem to be any evidence of "hybrid vigour" in our F_1 rabbits.

The F_2 has almost exactly the same range as that of the Polish stock. It is worth noticing that the slowest F_2 animals matured at 360 days,

whereas two-thirds of the Flemish stock matured at over 360 days and no less than 4 out of the 15 animals matured at 460 days. This failure of the very slowly maturing animals to reappear is, however, not inconsistent with some notion of inheritable factors determining the length of the growing period, because, as already noted, Q 73 matured ex-
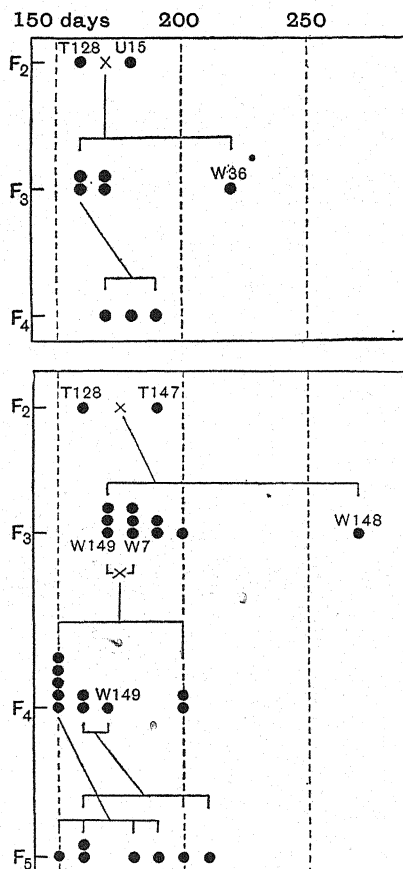
Fig. 21. Two families of rapidly maturing rabbits.

ceptionally rapidly and cannot be regarded as typical of the Flemish in this respect. On the whole, the F_2 distribution, with its obvious mode at 220 days, presents a quite usual appearance; it seems, curiously enough, as though it were a purer population than either of the parent stocks.

As nothing satisfactory about maturity seemed likely to emerge from a consideration of the weight material, I attempted to discover how far

it was possible to select from this mongrel F_2 population one or more strains relatively pure for some degree of maturity time. A start was made by trying to pick out a strain of rapidly maturing animals. Two ♂♂, T 128 (160 days) and U 102 (180 days), from the Polish-Flemish F_2 were mated to their sisters T 147 (190 days), T 150 (170 days), T 131 (190 days), T 132 (170 days), U 15 (180 days), U 103 (190 days) and U 116 (160 days), altogether 11 matings being made. The six matings with ♂ U 102 all showed considerable scatter and were therefore not continued. Of the five matings in which ♂ T 128 was the father, two seemed to breed tolerably true. The family from ♀ U 15 × ♂ T 128 is shown in Fig. 21, p. 301, and it will be seen that except for ♀ W 36 (220 days) all of the 8 descendants matured at under 200 days. For reasons concerned with another investigation, this family was abandoned in favour of that from the mating ♀ T 147 × ♂ T 128, whose pedigree is also shown in Fig. 21, p. 301. It will be seen that the 1st generation all matured rapidly except ♀ W 148, which was quite exceptional, maturing at 270 days. She was mated to her brother ♂ W 149 (170 days) and her slowness of maturing was clearly passed on to two of her three children. If we exclude ♀ W 148 and her descendants, and regard the pure strain as starting not from T 147 × T 128, but from W 7 × W 149, we are then left with a reasonably compact distribution, ranging from 150 to 210 days. Whether it is possible, under the roughly standardised conditions of our experiment, to narrow down further this range in the maturity time of this selected strain of rabbits, only continued breeding will show.

It was not till much later in our experiments that the attempt was made to select a slowly maturing strain. Six ♂♂ and six ♀♀ were picked out from our F_2 and F_3 material, and from these there is one mating which gives a progeny showing some promise of uniformity with regard to slow maturity. This family, *ex* Y 155 × W 93, is shown graphically in Fig. 22, p. 303, and though the numbers are still small, we have taken a sufficiently hopeful view to make a cross between one of these animals and one from the rapidly maturing strain with a view to studying the inheritance of maturity time.

While it is therefore likely that differences in maturity time can be attributed to genetic factors, we must not omit to consider other factors which probably play an important part in determining the growth of rabbits. Punnett and Bailey came to the conclusion that there was some slight correlation between weight and maturity time. Fig. 23, p. 303, shows the correlation curves for the present material, from which it is clear that both in the parent and in the cross-bred stocks the correlation

between heavy weight and slow maturing is well marked. Nor is this, after all, surprising, since obviously weight is the product of rate of growth and the number of days through which the animal continues to grow. Two points are worth noticing about the correlation curves: firstly, that the F_1 and F_2 curves are coincident, and secondly, that the
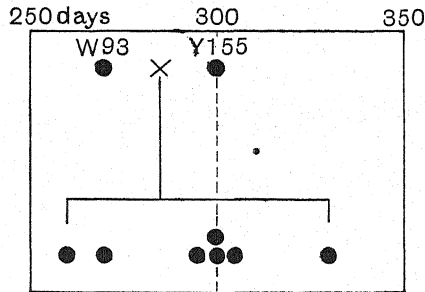
Fig. 22. A slowly maturing family.
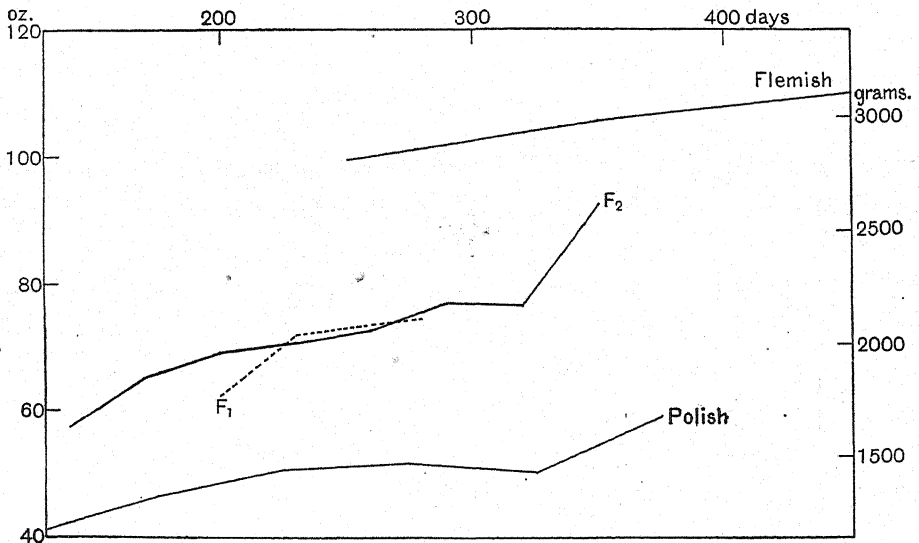
Fig. 23. Weight: maturity time correlation curves.

form of the F_2 correlation curve very closely follows that of the Polish stock. All this confirms the view already expressed, that if maturity time is determined by Mendelian factors, then we are all through this experiment dealing with an average mongrel population as far as this character is concerned. It might be suggested that this correlation is

TABLE VIII.

Weight: Maturity Time correlation (Polish-Flemish F_2).
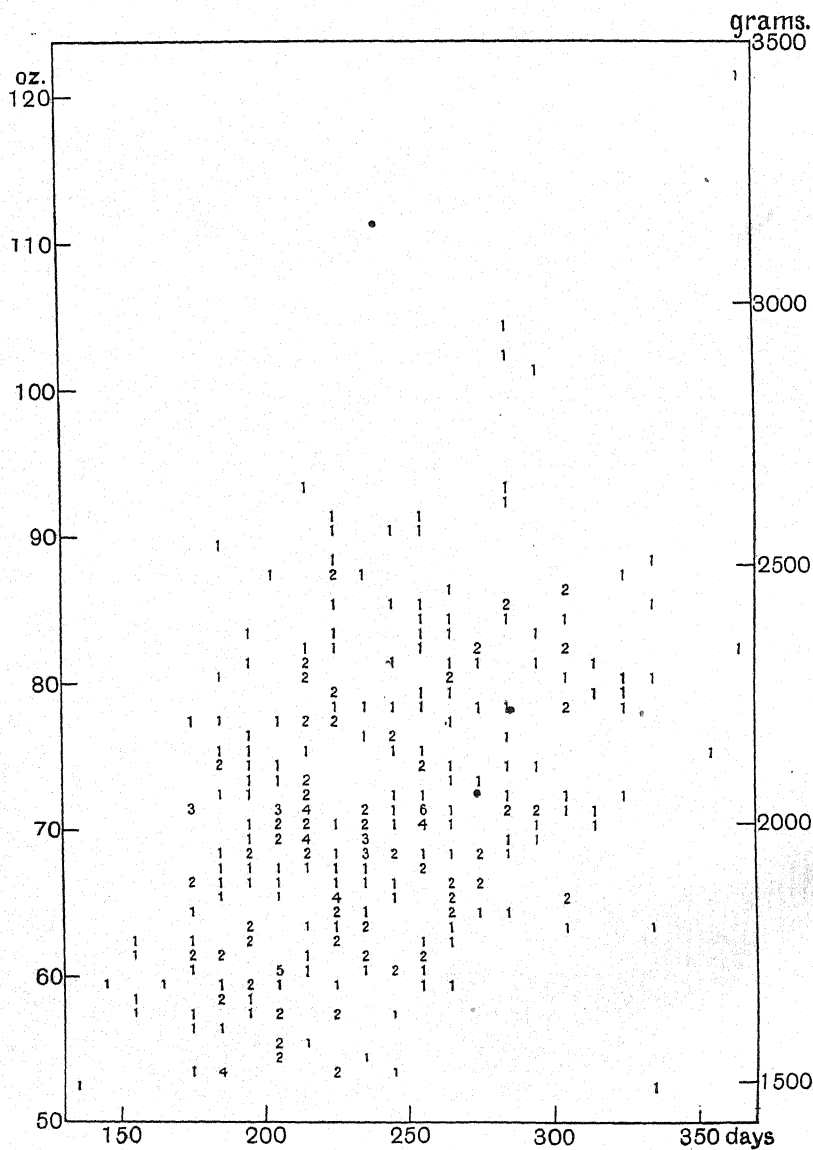
due to linkage between Mendelian factors. Our experiments, however, make this suggestion very unlikely, because in our original cross the lighter Polish parent ♀ Q 127 was slower to mature, taking 280 days to reach her Turning Point as against 210 days for the heavier Flemish parent ♂ Q 73.

A glance at the complete correlation table for the F_2 population (p. 304) shows that while on the whole there is association between heavy weight and slow maturing as against light weight and quick maturing, there is a fair smattering of animals for which this association does not hold. For example, the two lightest animals which weighed at maturity only 52 oz. each reached their Turning Points at 130 and 330 days respectively. And while, at the other end of the scale, it is true that nearly all the extremely heavy animals are slow maturers, yet there are five animals over 80 oz. which matured at under 200 days. This distribution of weight in relation to maturity time is in substantial agreement with the findings of Punnett and Bailey in their earlier experiment.

Since we have seen that the Turning Point is, in all probability, an indication of the oncome of puberty, it would not be surprising if there were a sexual difference in maturity time; but no evidence for this is discoverable in the material either pure or cross-bred.

The suggestion naturally arises that maturity time may be influenced, if not indeed determined, by seasonal conditions, but I was unable to find that either in the Polish or in the F_2 material the month in which the young are born has any appreciable effect on the maturity time, as Fig. 24, p. 306, shows. Those born in November and December seem to be somewhat slower in maturing (average maturity times 248 and 288 days respectively), a fact which might suggest that birth at an unnatural

TABLE IX.

Polish-Flemish F_2. *Season and Maturity Time.*

	Jan.	Feb.	Mar.	Apr.	May	June
Actual number of animals maturing	33	32	30	18	25	19
Average maturity time (days)	211	232	227	228	218	251
Weighted number of animals maturing	119	95	104	59	92	98
Weighted average maturity time (days)	207	234	234	232	213	258

	July	Aug.	Sept.	Oct.	Nov.	Dec.
Actual number of animals maturing	7	17	31	32	40	26
Average maturity time (days)	219	251	240	238	234	232
Weighted number of animals maturing	36	96	118	131	154	92
Weighted average maturity time (days)	204	253	237	250	235	239

The weighting has been effected by supposing 100 animals to be born in each month.

season of the year has a retarding effect on growth, were it not that the rabbits born in January mature the most rapidly of all (215 days).

It occurred to me that there might be a relation between the maturity time and the month in which the animal matured. But though I could find no evidence for this, one odd point emerged in the course of analysing the material, namely that relatively more animals reach their Turning Points in the autumn than at any other time of the year. This would seem to indicate that falling temperature is a condition which brings
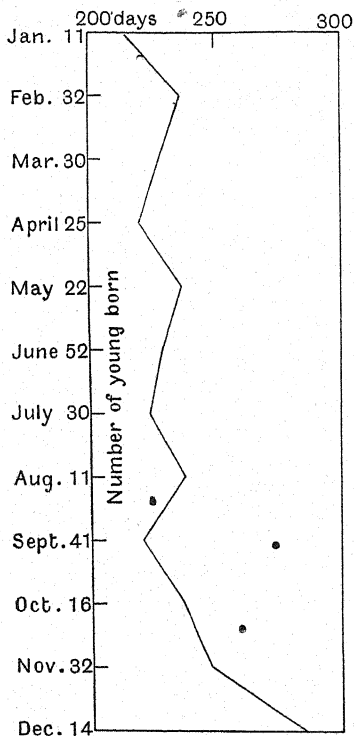
Fig. 24. Month of birth and maturity time.

about the Turning Point, a view which is borne out by the converse fact that relatively few animals mature in April, a month of rising temperature (Table IX). If the Turning Point were determined solely by the oncome of sexual activity, we should expect relatively more animals to mature in spring and relatively less in autumn, which is generally regarded as the period of least breeding activity. It must be pointed out, however, that our experience by no means bears out the commonly accepted view

that rabbits breed badly in the autumn and winter; the numbers given in Fig. 24, p. 306 show, it is true, that there are fewer births in December and January, but both November and February are months with high birth-rates, and September, which is regarded with extreme disfavour by the Fancy, has in our experiments produced more rabbits than any other month except June. The numbers are in any case small and the differences in question not great; all that can be said is that in view of the fact that there is evidence that the Turning Point represents the oncome of puberty, it is surprising not to find an excess of animals maturing during the season which is generally regarded as that of greatest sexual activity.

We may summarise our results as follows:

(1) It has been possible to select from the Polish-Flemish F_2 population two strains of rabbits, one of which matures on the average at 172 days and the other at 300 days. Though the numbers are so far small, these two families appear to breed true for this time difference.

(2) Under the conditions of the experiment, the maturity time is not appreciably affected by the season of the year in which the rabbits are born or by that in which they reach maturity.

(3) Maturity time is clearly not affected by sex and probably not by litter size.

(4) There is a considerable degree of correlation between heavy weight and slow maturing. But in spite of this, many rabbits have been bred in which this association is conspicuously absent.

4. PEDIGREE TABLE.

To show the Relationship of the Rabbits mentioned in the paper, and whose pedigrees are not given on the charts in the text.
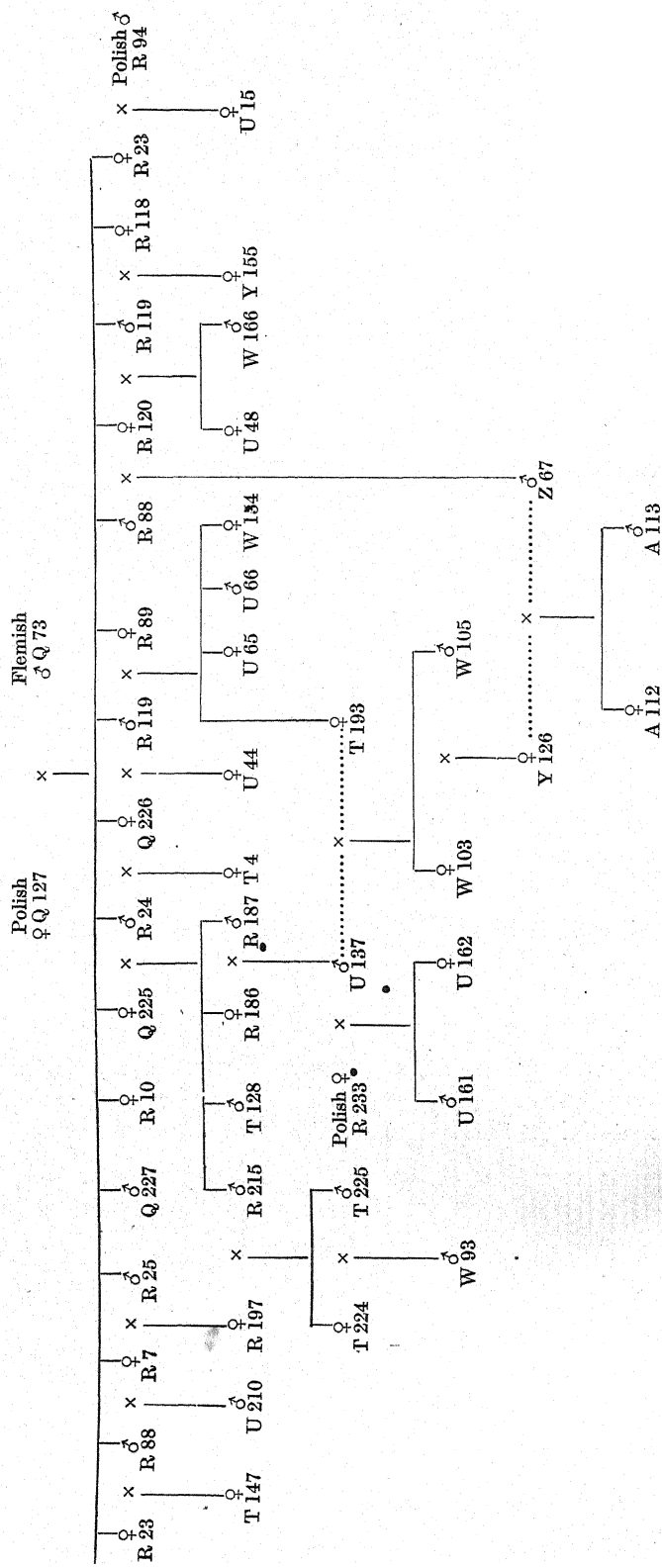
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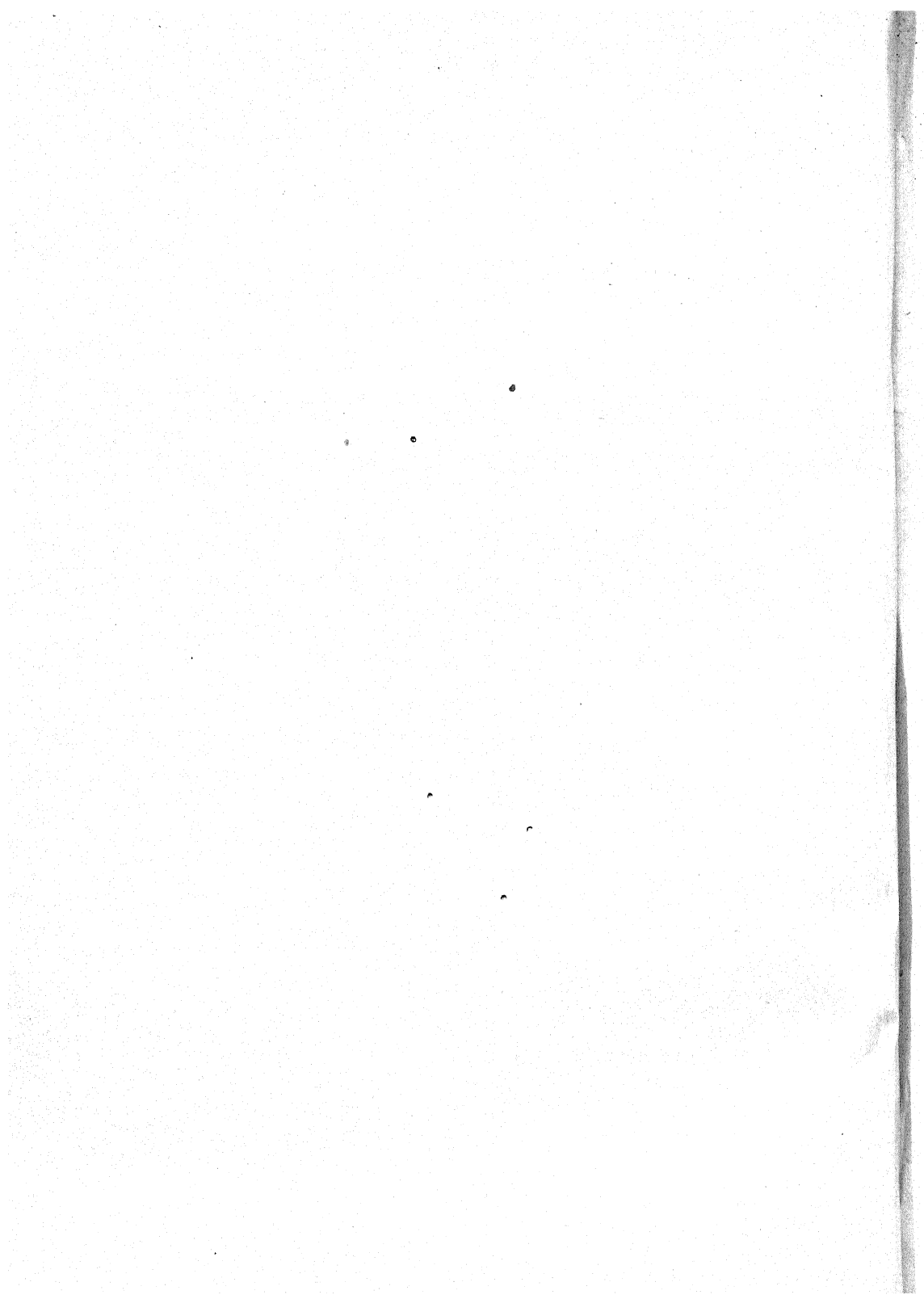
GENETIC STUDIES IN POTATOES: ABNORMAL SEGREGATION IN FAMILIES ARISING FROM THE CROSS *S. UTILE* × *S. TUBEROSUM*.

BY R. N. SALAMAN, M.D.

(With Four Plates and Nineteen Figures.)

CONTENTS.

INTRODUCTION.

SOLANUM UTILE has been grown as Barley for many years. Repeated efforts have been made to breed from it: between the years 1911 and 1926 the field pollination notes record 52 attempts to use it as a female in crosses with the domestic potato, of which 13, or 25 per cent., were successful; and 78 attempts in which it was employed as a male parent in the same series without a single success. The latter statement may be modified to this extent, that on one occasion a berry was formed but contained no seed. Numerous attempts were made to effect crosses between *S. utile* and other wild species: all of these failed with one exception. *S. utile*, again only fertile as the mother plant, was crossed by *S. chacoense*, the results of which will be detailed later.

Interspecific crosses with the tuber-bearing Solanums are, it is clear, not readily obtained. Paton (2, 3) records crosses between *S. maglia* and *S. edinense*, and between *S. maglia* and a Chilian cultivated variety, but obtained only one seed in each berry. He obtained a few seeds of the cross *S. commersonii* × *S. tuberosum* (= *S. utile*). From the description the hybrid appears to have resembled *S. tuberosum* in regard to

leaf and flower, and *S. commersonii* in regard to habit of growth—a result quite analogous to those to be described. After numerous failures, Wilson (9) crossed *S. commersonii* with a domestic potato and, like the writer, obtained a berry but no seeds. Robb (5) states that Wilson failed to obtain crosses with *S. polyadenium*, but did succeed with *S. maglia, S. commersonii* and *S. edinense*. The extent of the success he does not mention. With the last-named species, *S. edinense*, the writer has obtained several successful crosses, but as it is itself undoubtedly a hybrid, no evidence of interspecific fertility can be deduced. Prof. Knappe (4) has succeeded in using *S. jamesii* as the mother plant in a cross with a domestic variety.

The cross *S. utile* × *S. chacoense* may be very briefly dealt with. *S. chacoense* is an upright plant with many stems, a white stellate corolla, leaves with 4 pairs of lateral leaflets, and no folioles. The contrast between it and *S. utile*, which is described in detail a little later, is extreme. Numerous fertilisations resulted in one berry being formed, but it contained only one seed. This was planted and produced a plant indistinguishable in every way from *S. utile*; a flower of this latter plant was self fertilised under protection and a family of 100 individuals raised this year. The entire family consists of *S. utile* plants without the remotest indication of the *S. chacoense* grandparent.

CHARACTERS OF *S. UTILE*

S. utile is a distinct species of the tuber-bearing Solanums; it has been described under various names. In the *Botanic Garden*, vol. VII, 1837, it is described under the name of *S. etuberosum*—a name already misapplied to *S. edinense*. Seed sent to me under the names of *S. demissum* and *S. tuberosum* respectively has proved to be identical with it. I do not intend to discuss its origin or its relation to other species of the family. Suffice it to say that it is very distinct and reproduces itself by seed with unvarying uniformity.

The plants may attain 30–40 cm. but are more usually about 20 cm. in height. The habit of the plant is a spreading one, and the main stem has extremely short internodes (Pl. V, fig. 1). Later in the season the last two or three internodes may grow and thus increase the height of the plant, but in the earlier stages the main axis may exhibit a dozen or more nodes in a total length of 8–10 cm., whilst at the same time the plant in its cushion-like growth may form a circular mass with a diameter of 30–35 cm.

The stem is deeply pigmented reddish-purple and is very hirsute.
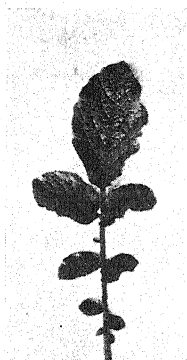
Text-fig. 1.

Text-fig. 2.

Text-fig. 1. A leaf of *S. utile* photographed whilst green.
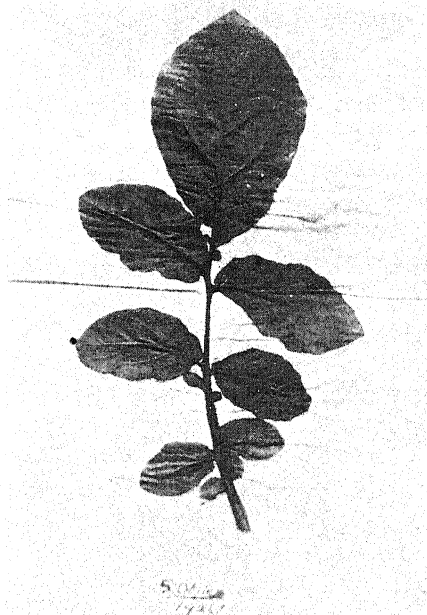
Text-fig. 2. A leaf of *S. utile* pressed.

Note both show the typical leaf characters: large terminal leaflet, blunt leaflets, wide spacing, sessile leaflets, angle of insertion greater than 80°.
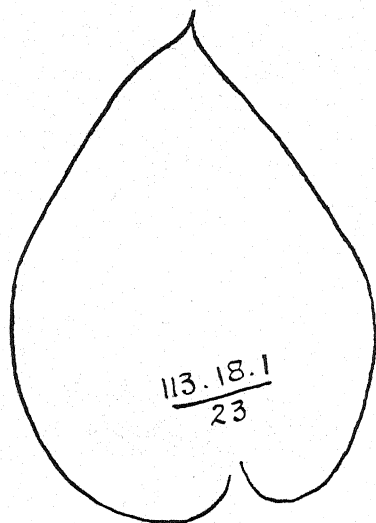
Text-fig. 3.

Text-fig. 4.

Text-fig. 3. Rubbing of a leaflet of 113.18.1 taken in 1923.
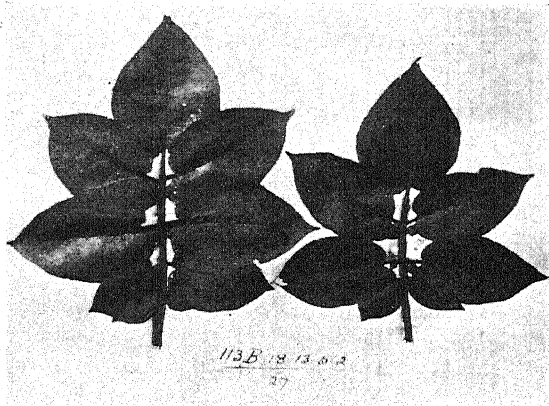
Text-fig. 4. Leaves of 113.18.13.5.2—one of the inbred domestic parents.

Flower-bearing branches occur from the lower nodes as well as from those higher up, and a characteristic feature is the formation of the oldest berries on lowly placed stalks which lie on the ground under cover of the oldest leaves.

The leaves are compound (Text-figs. 1 and 2) and are composed of a very large terminal leaflet followed by two or at most three pairs of lateral leaflets. Minute folioles may be found between the leaflets, but their presence is not constant and in all cases their development is very restricted.

The leaflets are attached to the petiole either by an extremely short and scarcely recognisable independent stalk, or the leaf tissue actually passes into that of the petiole as a wing-like extension. Similarly the terminal leaflet fuses by wing-like extensions into the petiole.

The actual shape of the leaf is peculiar and is differentiated from all domestic varieties by the obtuseness of its apex. In some cases the leaf may end in an almost unbroken semicircular line; in others, a very slightly developed but blunted apex is to be seen.

The surface of the leaf is naturally finely rugose, a rugosity quite distinct from that common in virus disease of the potato, and is beset with very coarse hairs.

The flower of *S. utile* is of the same type as that of the domestic potato inasmuch as its corolla is rotate and its calyces are mucronate, though their pointed terminal processes are always quite short. It differs from the domestic varieties in the colour of the corolla being a deep violet, the greater part of the pigment of which is deposited on the lower, *i.e.* under layers of the mesophyll. In the domestic series, colour occurring in positions other than the central vein of the petal is invariably on the upper surface.

The anthers are small and very delicate, arranged in a compact and narrow cone, contrasting strongly with the stouter, broader, and often very irregular cone found in the domestic potato. They are charged with abundant and for the most part perfect pollen grains.

The style is fairly long and terminates in a small, round, unnotched stigma.

The berry is cordate: when young it is very definitely long and pointed, and a more or less definite longitudinal sulcus occurs on either side, as the berry enlarges this tends to disappear and the shape becomes cordate. The surface of the berry is green and is marked by numerous small white spots.

The roots of the plant do not differ materially from those of the

domestic plant but the stolons, on the other hand, are very numerous, extremely thick, and spread widely in all directions, often coming to the surface and giving rise to another plant, so that daughter plants may be found as far as 2 metres away from the original parent. Tubers are very scarce and occur as thickening of the stolons. Neither in Barley nor Cambridge have they ever attained a size larger than about 2 cm. in length. They are pure white. The stolons, however, persist over winter in the soil and in the following year will give rise to a host of secondary plants.

The domestic parents used in the crosses were seedlings of a very much inbred family (see Text-fig. 5). In form they did not differ from the ordinary potato; the flowers were white, the stolons short, and the tubers elongated and colourless.

Certain characters common to them and to all the domestic series need recognition.

The plants are much taller than *S. utile*, the internodes of the stem being much longer. The flower stalks are borne on the top of the plant, and any berries that may be formed are to be found hanging from the uppermost parts of the adult plants and not, as in *S. utile*, hidden away at the base.

The leaves are compound; the terminal leaflet, though generally larger in area than the laterals, is not so overwhelmingly so as in *S. utile*. The number of laterals in a well-developed leaf is never less than three, and often four; the intervening folioles are very variable in their development; there may be two pairs between each pair of laterals and they may attain such size as to fill in all the available space between the leaflets, so that the whole leaf presents a more or less complete surface (see Pl. VII, figs. 1–4). The folioles when least developed in the domestic varieties attain a greater size and prominence than in *S. utile*.

Conspicuous differences between the domestic potato and *S. utile* are to be found in the shape of the leaflet which, in the former, is invariably sharp and more or less acutely pointed at the apex, and at the base complete in itself, ending abruptly in a well-defined stalklet which arises sharply from the main petiole. The length of the independent portion of the leaflet petiole, the angle at which it is inserted into the main petiole, no less than the shape of the apical portion of the leaflet, vary widely from variety to variety and, to some degree, even within the variety; but in no case, as in *S. utile*, are the leaflets sessile or the apices blunted.
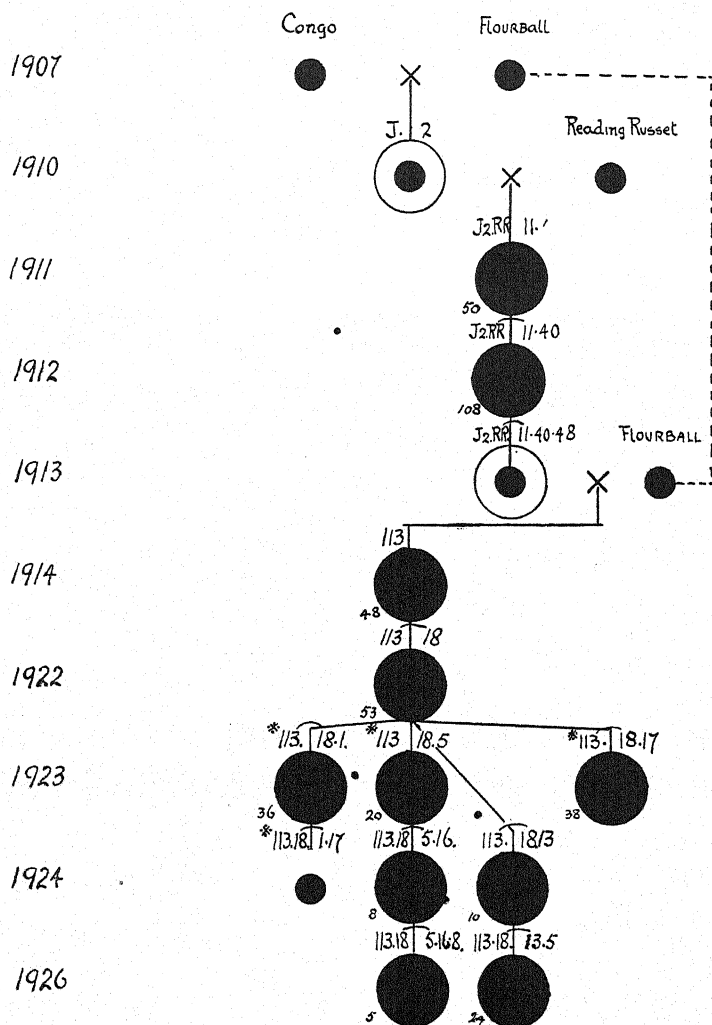
Text-fig. 5. The pedigree of the domestic parents used. The numerals above the figures in the diagram indicate the pedigree number of the family; those below, the number of plants contained in it.

● = a family of plants of the normal domestic type.

● = an individual plant.

◉ = an individual plant from a selfed family.

✳ plants so marked have been used as male parents.

F_1 EX *S. UTILE* × DOMESTIC.

The F_1 generation of *S. utile* by domestic, of which five families arising from five distinct male parents have been raised, is completely uniform, and as regards their morphological characters the individuals of any one family cannot be distinguished from those of any other. In two of these families, however, where the male parents were quite unrelated, a very distinct difference occurred, in respect to the exhibition of the symptoms of simple mosaic, the one family showing but the merest trace, the other a uniform and very considerable mosaic mottling.

An F_1 individual plant, no matter what its domestic paternal parent, starts with a form of growth, low and spreading, closely resembling the *S. utile* parent. After a time, the internodes lengthen and the plant attains a height of 60–70 cm.; later, the stout stems overborne by the wealth of foliage, tend to collapse, and at the end of the season the main stems when outstretched attain an average length of about 120 cm., but stems 150 and even 180 cm. long are not infrequent.

The stems are heavily pigmented, and the tone of purple is the same as that seen in *S. utile*.

Flower-bearing pedicels are distributed all over the plant (Pl. VI) and are especially numerous as in the domestic plant at the higher nodes, but they occur also low down as in *S. utile*, so that in this respect the habit of both parents is represented in the F_1 plant.

The leaves, both in regard to their conformation and the shape of their component leaflets, occupy an intermediate position between the two parents. There are always three, and sometimes four, pairs of leaflets, and the terminal leaflet is not much bigger than the first laterals, characters both common to the domestic plant.

The leaflets are inserted into the main petiole by means of a short independent stalk, and their basal ends do not merge into the tissues of the petiole as in *S. utile*. The apex of the leaflets is invariably pointed as in the domestic parent, and though the angle is not often as acute, it readily differentiates the F_1 leaflet from the *S. utile* parent leaflet. The texture of the leaflet is hairy and finely reticulated and in these respects resembles more closely *S. utile*.

Folioles are always present and may attain a considerable development, though rarely to the same extent as in the domestic plant. The flower of the F_1 plant is twice the size of that of *S. utile*, and rather bigger than that of most domestic plants. Its colour is the same in all

the F_1 plants, a bluish-heliotrope. The under surface of the corolla is of a deeper colour than the upper, corresponding to violet-purple (192.1 of the Société Française des Chrysanthémistes colour chart), whilst the upper surface corresponds to lavender-blue (204.3) of the same. The anthers, style and stigma all resemble those of *S. utile* but are larger in size. The flowers set freely, and enormous crops of berries may be found hanging from pedicels springing from the base to the apex of the stem. The shape of the berry is cordate and rather more obtuse than that of *S. utile* but, like it, freely covered with fine white spots.

The root system is very extensive and the stolons are both numerous and long; they are not quite so thick as those of *S. utile*, but like them they wander off in all directions and throw up secondary plants at a distance. Tubers are borne near the plant; they are irregular in shape, more or less elongated and with a lateral depression which is similar to that seen in the tubers of *S. etuberosum*. The colour is white, though frequently there may be developed a purple tinge; the eyes are deep. The crop is small.

Although the F_1 plant is intermediate in most of its characters, when seen growing *en masse* its affinities with *S. utile* are perhaps the more striking. The vigour of its growth and the luxuriance of its beautiful flowers, the finely rugose matt surface of its leaves no less than the dusty olive-like colour of its foliage, proclaim it a thing apart.

EXCEPTIONAL F_1 PLANTS.

Although emphasis has been laid on the striking uniformity of the F_1 plants, there were in one family (365 *B*) three plants (Nos. 10, 13 and 15) in which the dominance of the *S. utile* parent was much more impressive than in any of the others. These three plants were all alike; they were lower in stature, viz. 60 cm. instead of 120 cm. or more.

The colour of the flowers was exactly the same deep blue-purple which is peculiar to *S. utile* and their foliage resembled, as far as was observed, that of *S. utile*. Rubbings were taken of 10 to 20 first left lateral leaflets of each of these plants, as well as a similar number of the sister plants Nos. 1, 2, 3, 5, 6, 8 and 16, for the purpose of determining the Leaf Index (4). The rubbings from Nos. 365.10, 365.13 and 365.15 are all precisely similar and resemble those taken from *S. utile*; most characteristic is the absence of any apical process, the distal margin of the leaf is either in the form of an obtuse angle or of a semi-circle (see Text-fig. 10, p. 324). The rubbings from the sister normal F_1 plants are shown in Text-figs. 8 and 9, p. 324; in every case the leaflet is
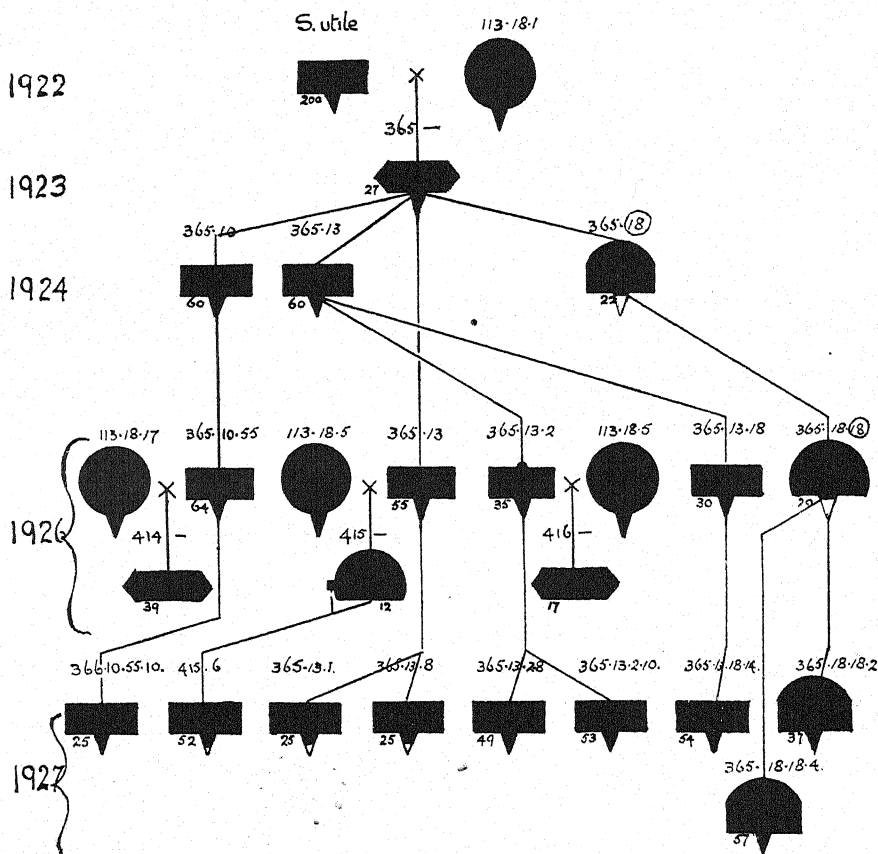
Text-fig. 6. The pedigree of the 365 group of hybrid families. The numerals above the figures in the diagram indicate the breeding number of the family; those below, the number of plants contained in it.

▼ = the presence of naturally-formed seed-balls on all individuals; a partially filled ▽ implies that only a proportion of the plants bear berries.

● = a family of plants of the normal domestic type.

▬ = a family of similar individuals whose morphological characters are identical with those of *S. utile*.

⬢ = an F_1 family whose members are perfectly uniform, their characters approaching the wild rather than the domestic type.

◗ = a family of unlike seedlings whose habit, foliage and flower colour approximate to the domestic type.

◖ = a family of unlike seedlings whose characters resemble the domestic type yet retain in some individuals characters peculiar to *S. utile*.

ˑ ▪ ▮ = the occurrence of a seedling of domestic or *S. utile* type respectively in a family otherwise intermediate in character.

bigger and is furnished with a quite definite though not long apical process.

The Leaf Indices of the aberrant F_1's, viz. Nos. 10, 13 and 15, are 59, 60 and 59 respectively; those of the normal F_1's are 58, 58, 58, 57, 61, 58, 58, and that for *S. utile* = 61.

The three abnormal F_1 plants grew to a height of 2 ft. and when harvested each bore about 2 oz. of tubers. This at once differentiated them from the normal *S. utile* which bears but a minute tuber, if any at all. These tubers were carefully preserved, and two plants of each were grown in the following year, 1924. They grew uniformly to 5 ft. in height and bore large, pale blue flowers identical with those of the normal F_1's, *i.e.* unlike those borne by them in the previous year or those of *S. utile* itself. The leaves of these plants were photographed living (see Pl. III, figs. 6 and 7) and were rubbed for the Leaf Index (see Text-figs. 11–13, p. 327). The plants bore crops up to 1 lb. in weight.

Pl. II is a photograph of 365.10 taken in the later mutated condition in 1924, and represents in all its details a typical F_1 plant of the cross *S. utile* × domestic potato.

In 1925, 365.10 was regrown and its characters, as observed in 1924, were faithfully reproduced. In all the photographs and rubbings of the leaflets of the regrown plants, a well-developed apical process is seen. The Leaf Indices have remained about the same. The new values are 60, 59 and 58, as compared to 59, 60 and 59—differences which are not significant.

In the writer's experience of the potato plant, no other case of a mutation affecting a large range of characters at one time has been met with, though a mutation in the bud tissue of a tuber eye may certainly involve more than a mere tuber skin colour variation (8). It is of interest that the somatic "mass" segregation under discussion was followed by a similar gametic mass-segregation. In the tuber mutation referred to, where a loss of colour in the tuber and a change of shape in the leaflet resulted from a mutation, it was demonstrated that a parallel change had taken place in the gametes. In the present case the phenomenon is similar but on a bigger scale. The facts, however, in this case were so striking and surprising that the possibility of error naturally suggested itself and has been carefully considered. Revision of all the notes over the three years in which these plants were grown, discloses no evidence of any mistake. The three F_1 plants, though looking so completely like the wild *S. utile*, yet differed, it was noted, in two characters; they were considerably taller than the wild plant, and they bore several tubers of moderate size.
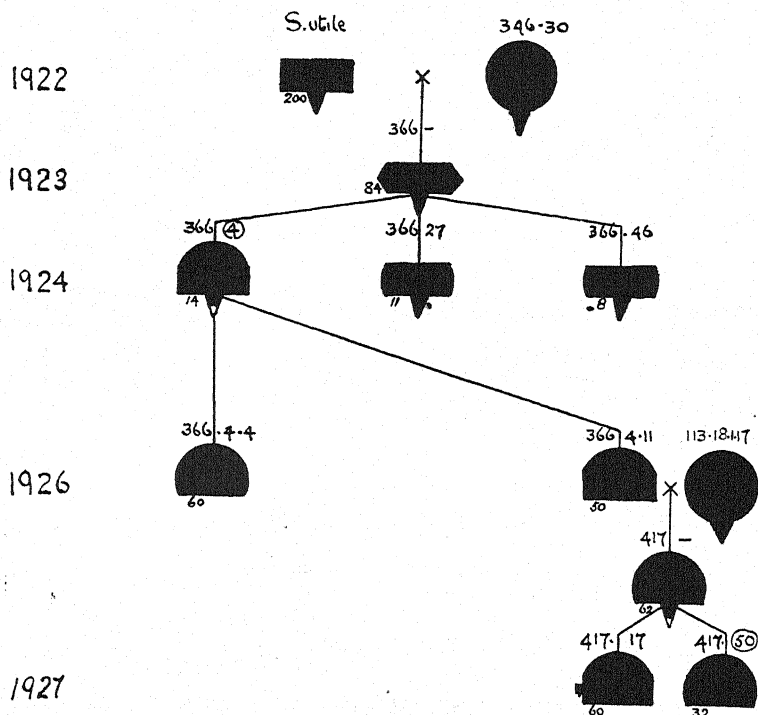
Text-fig. 7. The pedigree of the 366 group of hybrid families. The numerals above the figures in the diagram indicate the pedigree number of the family; those below, the number of plants contained in it.

= the presence of naturally-formed seed-balls on all individuals; a partially filled ∇ implies that only a proportion of the plants bear berries.

= a family of plants of the normal domestic type.

= a family of similar individuals whose morphological characters are identical with those of S. utile.

= a family of individuals similar to the above except that the shape of the berries segregates into "rounds" and "longs," and in some there is also a segregation of flower colour.

= an F_1 family whose members are perfectly uniform, their characters approaching the wild rather than the domestic type.

= a family of unlike seedlings whose habit, foliage and flower colour approximate to the domestic type.

= a family of unlike seedlings whose characters resemble the domestic type yet retain in some individuals characters peculiar to S. utile.

= the occurrence of a seedling of S. utile type in a family otherwise intermediate in character.

Although the plants were harvested by the writer, the possibility of the tubers being really those of a neighbour—the stolons in this family are very long—may be ruled out, because it is highly improbable that the same mistake would have been made on three plants of the same family at the same time. It is further recorded in the 1923 notes that the stolons of Nos. 13 and 15 were under 18 in., hence the tubers must have been borne not unduly far from the stem. The stolons of the remaining F_1's were much longer.

It is unfortunate that no cytological examinations were successful on any of these three plants, but those on the other F_1's show a disturbed condition of the chromosomes as will be described later. There seems no reason to doubt that we have in the case of these three F_1 plants a genuine example of a somatic segregation involving a whole group of characters. This conclusion is further borne out by evidence showing that the progeny which arose from the two of these abnormal F_1 plants, whose inheritance was studied, viz. 365.10 and 365.13, exhibited a very disordered genetic composition and that, notwithstanding the existence in subsequent generations of whole families apparently pure for the *S. utile* character (see Text-fig. 6), breeding experiments demonstrated that many specifically domestic characters were lying latent in the majority of the gametes. Convincing proof of this is to be found in the individual No. 18 of family 365.13.2 (Text-fig. 14, p. 331), a family of 35 plants directly descended from 365.13 in which 34 plants were exactly similar to *S. utile* whilst one, No. 18, had several "domestic" characters in both flower and leaf (see Text-fig. 15, p. 331) which it could only have acquired from its domestic great-grandparent.

LATER GENERATIONS FROM EXCEPTIONAL F_1 PLANTS

The subsequent breeding with which this communication deals, refers in part to families raised from two of these F_1 abnormal individuals.

In Text-figs. 6 and 7 are shown the pedigrees of the families dealt with. It should be noted that, unless mention is made to the contrary, all the families discussed have been raised by hand-fertilisation and the utmost precautions have been taken to prevent cross-fertilisation by insect or wind.

Three of the 27 plants of the F_1 family 365 B were selfed and their progeny followed through two further generations. Two of these F_1's were 365.10 and 365.13, whose likeness to the wild parent *S. utile* in 1923 and subsequent mutation has been referred to.

The F_2 families (see Text-fig. 6) arising from the F_1 family 365 were:

365.10 (number of plants 60). A perfectly uniform group of plants identical with the grandparental *S. utile*, and all bearing cordate berries.

365.13 (number of plants 115). A family of plants which, like 365.10, is identical in all respects with *S. utile*. A fresh batch of seed of this family was grown in the following year and behaved in a similar manner.

365.18 (number of plants 22). In this family, segregation of foliage, flower colour characters and habit occur. Neither the wild nor domestic parent are reproduced in their entirety. One individual, No. 18 (see later), is in appearance exactly like *S. edinense*. Berries are very scarce. This family arose from unprotected selfed berries.

From these F_2 plants were raised F_3 families, viz.:

From 365.10 arose 365.10.55 (number of plants 64). A uniform family reproducing *S. utile* in all its characters.

From 365.13 arose 365.13.1 (number of plants 25). A family that is perfectly uniform and resembles *S. utile* in all respects.

From 365.13 arose 365.13.2 (number of plants 35). All individuals were identical with *S. utile* except one seedling, No. 18, in which segregation of both flower colour and leaf character was evident, and with a habit of growth resembling that of the domestic parent. It was unfortunately sterile.

From 365.13 arose 365.13.8 (number of plants 25). A family that is perfectly uniform and resembles *S. utile* in all respects.

From 365.13 arose 365.13.18 (number of plants 30). This family reproduces *S. utile* in all characters.

From 365.18 arose 365.18.18 (number of plants 20). Segregation of all characters including those of cropping occurs in this family. No plants resemble or approach at all closely to *S. utile*; all, on the contrary, approximate to a greater or lesser degree to the normal domestic type of growth. This family arose from unprotected selfed berries.

From the F_3 plants, F_4 families were raised in 1927, viz.:

From 365.10.55 arose 365.10.55.10 (number of plants 25). All identical with each other and with *S. utile* in all characters.

From 365.13.2 arose 365.13.2.8 (number of plants 49). All identical with each other and with *S. utile* in all characters.

From 365.13.2 arose 365.13.2.10 (number of plants 53). All identical with each other and with *S. utile* in all characters.
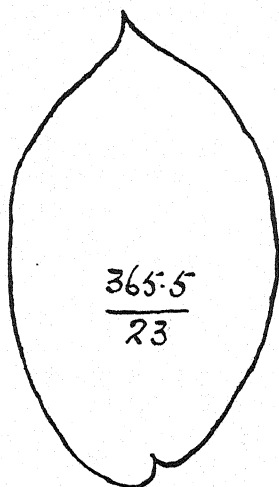
Text-fig. 8.
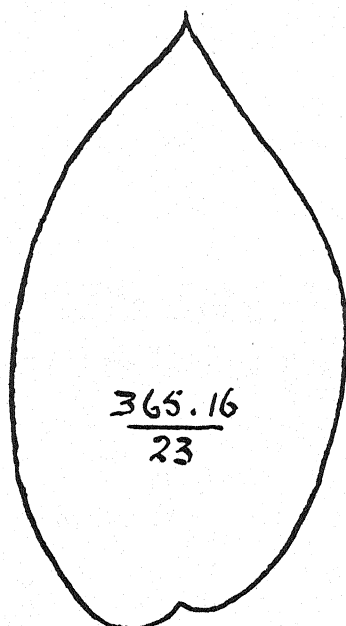
Text-fig. 9.

Text-figs. 8, 9. Rubbings of the first left lateral leaflets of normal F_1 plants Nos. 5 and 16.
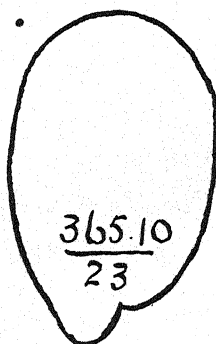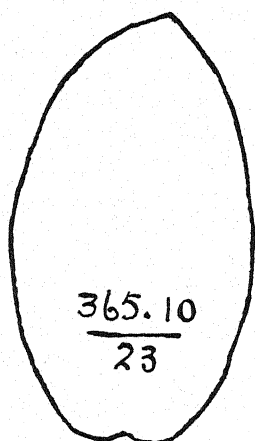
Text-fig. 10. Rubbings of the abnormal F_1 plant No. 10 in 1923.

From 365.18.18 arose 365.18.18.2 (number of plants 37). A weakly
family in which the character of habit of growth and leaf shape are
predominantly domestic.

From 365.18.18 arose 365.18.18.4 (number of plants 57). A family
similar in all respects to 365.18.18.2.

From the F_2, F_3 and F_4 families so far described, it is seen that those
springing from two of the F_1 plants, viz. Nos. 10 and 13, reproduce in
all their members exclusively the character of the wild grandparent
S. *utile*, with the single exception found in one F_2 family (365.13.2),
where one plant exhibited some characters of the domestic grandparent.
In all, these 9 families contained 451 individuals of which 450 were
identical with each other and with the original wild grandparent, whilst
but one plant showed evidence of the existence of any other ancestry. The
practical elimination of one side of the ancestry in these families displays
a peculiar segregation occurring in the male and female germinal tissues
of some of the F_1 plants; there is in fact a mass segregation of characters
peculiar to a well-established specific type. A similar mass segregation
of characters we have just seen occurred as a somatic mutation in Nos.
10, 13 and 15 of the F_1 family No. 365. Whatever may eventually prove
to be the chromosomic basis of this mass segregation, it is clear from
the evidence derived from certain back-crosses which were made on F_3
individuals of apparently completely wild appearance that some of the
domestic characters, presumably residing in supernumerary chromosomes
of domestic origin, are still present though latent. From one F_1 plant,
viz. No. 18, a selfed family was raised in which a quite different and
possibly a more or less normal segregation of characters has taken place.
In this family we find tall plants with long internodes, and dwarf plants
with the crowded nodes peculiar to S. *utile*; some with pointed, long-
stalked leaflets with or without folioles, and others with sessile blunt
leaves and no folioles; some with the deep bluish-purple flowers of
S. *utile*, others with white or pale blue flowers; some with the round
berry of the domestic potato, others with the long or cordate berry of
the wild S. *utile*. In some cases the S. *utile* type was reproduced almost
in its entirety, in others the domestic; but in no case was the parental
type quite perfect. In the F_3 family 365.18.18 derived from the above,
the segregation of characters follows the same lines, but the individual
plants exhibit on the whole more of the domestic characters in com
bination than they do of the wild; indeed, whilst one or two plants
differ only from ordinary domestic potato plants in bearing their flower
colour on the lower surface of the petal instead of the upper, none

exhibit the wild type of character so uniformly as to pass for *S. utile* even on a casual inspection.

Two F_4 families of this group, viz. 365.18.18.2 and 365.18.18.4, were grown at Ormskirk to test their susceptibility to Wart Disease. Owing to the fact that the condition of climate and soil were inimical to seedlings, and that every one was susceptible, their growth was but poor, and it is only possible to record that in both families, 94 individuals in all, the leaves were either intermediate or distinctly domestic in type, and that the plants were upright and tall in habit, and that none resembled the *S. utile* parent.

If now we return to the back-crosses which were made on one of the F_2 and two of the F_3 plants with close relatives (see Text-figs. 5 and 6), of the original inbred "domestic" parent, we find that two quite distinct and unlike results were obtained.

In the crosses 365.10.55 × 113.18.17 and 365.13.2 × 113.18.5 the 365 derivative parent in each case presented every characteristic of the *S. utile* great-grandparent. The resultant families, viz. 414 and 416, Text-figs. 6 and 16, were absolutely uniform and identical with the original F_1 in every respect except one, for whilst the plants of the F_1 families were smothered with berries, not one of the 56 individuals comprised in these two back-cross families bore a single berry. On the other hand, both families displayed the extraordinary vigour (heterosis) so well seen in the original F_1. No corresponding increase of vigour was observed in the family 415 described below.

The third back-cross family, viz. 415, from the cross 365.13 × 113.18.5, yielded a still more anomalous result. It may be remembered that 365.13 was itself a family with 115 individuals, all of which were pure *S. utile* in type, and from this family three F_3's were obtained, viz. 365.13.1, 365.13.2 and 365.13.8, in which all plants were again of *S. utile* type.

It will further be seen on referring to the genealogical tree (Text-fig. 6) that the two F_4 families, 365.13.2.8 and 365.13.2.10, all directly derived from this same 365.13, the parent of the cross 415 under consideration, produced also pure *S. utile* families. It might therefore have been thought that 365.13 would, notwithstanding the fact that it is itself an F_1 plant, produce male and female gametes containing only *S. utile* characters, and that when back-crossed by the "domestic" parent it would once more reproduce the distinct F_1 type which has already been described in detail.

The back-cross 415, which contained only 12 individuals, contradicts
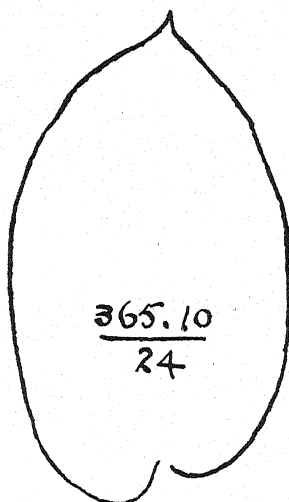
Text-fig. 11. Rubbing of the same plant, F_1 No. 10, taken in 1924 after it had mutated.
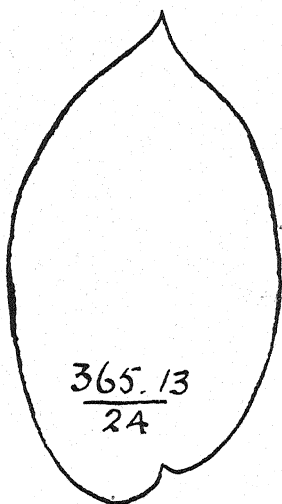
Text-fig. 12.
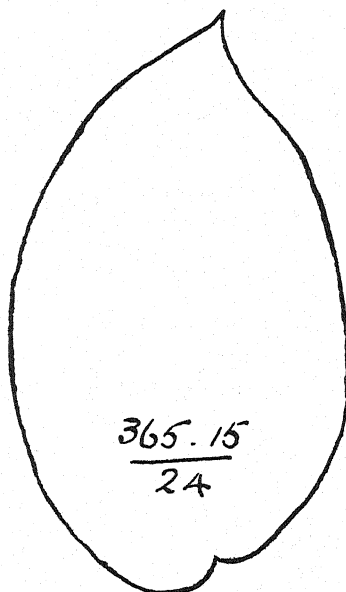
Text-fig. 13.

Text-figs. 12, 13. Rubbings of the F_1 plants, Nos. 3, 13 and 15, in 1924 after they had mutated.

such a view, for it displayed segregation of leaf and flower characters, and all its members except one resembled the domestic type much more closely than the wild one. None, however, bore any berries. The exceptional plant, No. 6, reproduced the *S. utile* parent in every particular, including that of bearing cordate berries. From this individual wild type plant an F_2 family, 415.6, was raised and all of its plants once more faithfully reproduced the *S. utile* type. In the next series of crosses a somewhat similar event occurred.

ANOTHER *S. UTILE* × DOMESTIC CROSS.

A further cross, *S. utile* by a domestic seedling, 346.30 (an F_1 offspring of the cross Nithsdale by Edzell Blue), was made, and the families derived from it are diagrammatically pictured in Text-fig. 7.

The F_1 family 366, of which 84 plants were raised, was perfectly uniform and resembled in all respects the F_1 family 365 already described; there was an abundance of berries on all plants. This resemblance is of interest when one remembers that in the 365 family the domestic parent was a much inbred plant, homozygous for most of its characters, whilst here the domestic parent is an F_1 and heterozygous for most characters. In 365 the leaflets of the domestic parent were broad (see Text-figs. 3 and 4); in this case they are noted as being long and narrow. The two F_1 families showed no evidence of any distinction of a morphological nature which could be referred to either domestic parent. There was, however, one difference between the two families, and that was that 366 showed the presence of mosaic disease throughout the season, whilst in 365 it was only towards the end of the season that some mottling was to be seen.

Three F_2 families were raised from the F_1 366:

366.4 (number of plants 14). Segregation of characters affecting habit, leaf shape and flower colour were found, but the plants, though never completely domestic in type, showed more affinity to the domestic parent than to the wild.

366.27 (number of plants 11) and 366.46 (number of plants 8). Both these families closely resembled each other and the *S. utile* grandparent. They differed only from the latter by the occurrence of segregation in the berry shape, long in some plants and round in others. The flowers in shape and type were wild and, except for one individual in 366.46 which bore white flowers, they were of the deep blue colour characteristic of *S. utile*.

Two F_3 families were raised from 366.4:

366.4.4 (number of plants 60) and 366.4.11 (number of plants 50). The *S. utile* type was not reproduced at all; nor was the domestic, that is to say, in its entirety; all the plants, however, approximated much more nearly to this type than to that of the wild.

A back-cross family, 417, obtained by crossing 366.4.11 with 113.18.1.17, produced a family of 62 individuals in which all the individuals more or less closely resembled the domestic parental type, and in which segregation took place freely as regards characters such as flower colour and berry shape and the development of folioles; whilst leaflet shape, habit of growth and length of internodes, as in all such families, followed the domestic type.

From the back-crossed family, two others were raised, one of which, 417.50, containing 32 plants, reproduced the characters of 417 more or less exactly, whilst in the other, 417.17, containing 60 plants, the approximation of most of the plants to the domestic type was rather less close. It is in this family that there occurred one plant of absolutely pure *S. utile* type; a similar occurrence was noted in the back-cross family 415 belonging to the series previously described (cf. p. 326).

The *S. utile* plant, No. 59, of the 417.17 family, though indistinguishable from the pure-line plants of the wild type, has exhibited a physiological distinction to these latter. *S. utile* plants are very late in maturing. Grown in Barley for the last 15 years they have shown themselves practically immune to the attacks of *Phytophthora infestans*: this plant, however, as early as 24 August 1927 was almost destroyed by blight.

One other of the derived *S. utile*-like families, viz. 365.13.18.14, has also been attacked by blight, but in this case there appeared to be (24 August 1927) a very definite segregation between immune and susceptibles; 45 of the former are quite untouched; 7 of the latter are almost destroyed.

LEAF CHARACTERS.

Mention has already been made of the essential differences between the leaves of the wild *S. utile* and the common domestic potato: it will be necessary here to go rather more fully into details. The following are the characters which distinguish the two original parental types, and which appear to be capable of more or less independent segregation.

Character	S. utile	Domestic
Distal extremity of leaflets	Either semicircular or ending in an obtuse angle, not surmounted by an apical process	No matter whether the end is elongated or blunt, it s always surmounted by a sharp more or less elongated apical process
Shape of the distal portion of leaflet	Blunt	More or less acute
Size of terminal leaflet	$1\frac{1}{2}$ to 3 times the area of that of the first lateral	In general, equal to or $1\frac{1}{2}$ times the area of the first lateral
Angle of inset of lateral leaflets to the main axis of leaf	Varies from 80° to about 110°, usually 90°	Varying, but usually from 45°–60°
The distance between the insertion on the main axis of the first and second pair of laterals	Is considerably more than one-half the length of the petiole and axis combined of the first lateral leaflet	Is distinctly less than one-half, often one-third the length of the petiole and axis combined of the first lateral leaflet
The insertion of the terminal leaflet on the midrib	The sides of the leaflet merge more or less gradually with the midrib	The sides of the leaflet end in semicircular wings which end abruptly in the midrib
Relation of lateral leaflet to midrib	The lateral leaflets are sessile and their basal edge may merge with the midrib	The lateral leaflets are stalked
Presence or absence of folioles	Often absent: never numerous	Always present on adult leaves, often numerous
Size of folioles	Always small—never more than about one-fifteenth of the area of the first lateral leaflet	Variable in size on the same leaf, the largest often as large as one-fifth of the area of the lateral leaflet
The texture of the leaflet	Coarse; the smaller veins well marked with elevated areas between them	Soft, and more or less smooth, the smaller veins less well marked and the intervening tissue less elevated

The characters of the *S. utile* leaf have been already described, and it is not necessary to do more than refer to figures where the fresh and the pressed leaf are shown.

The domestic parent used in the 365 series of families is represented by Text-fig. 3 which is a tracing of a typical first lateral leaflet of an adult leaf of the original domestic parent 113.18.1, and by Text-fig. 4 a photograph of the pressed leaf of the much inbred derivative of the same, viz. 113.18.13.5.2. Pl. VII, figs. 1–4 are typical leaves of the domestic varieties Mr Bresee, Great Scot, Leinster Wonder and Sharpe's Express.

The leaf characters of the "domestic" parent used which contrast most acutely with those of *S. utile* are:

1. The terminal leaflet is only a little larger than the first lateral.
2. The apical angle of the leaflets is elongated and sharp.
3. The leaflets are placed closer together in the main axis.
4. The leaflets are stalked and there is no merging of the base of the leaflet into the main axis of the leaf.
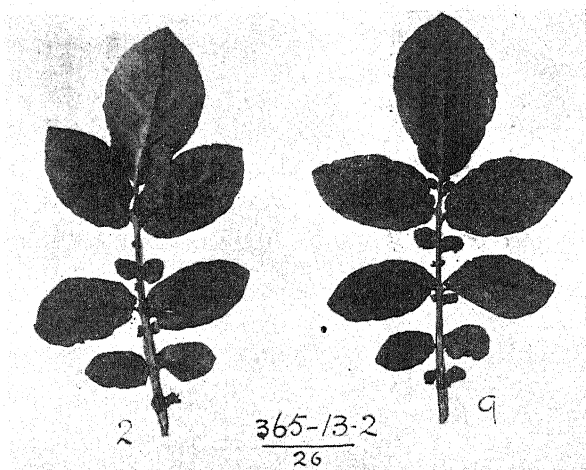
Text-fig. 14. A leaf of 365.13.2—a plant of a family which reproduced the *S. utile* type in all its characters.
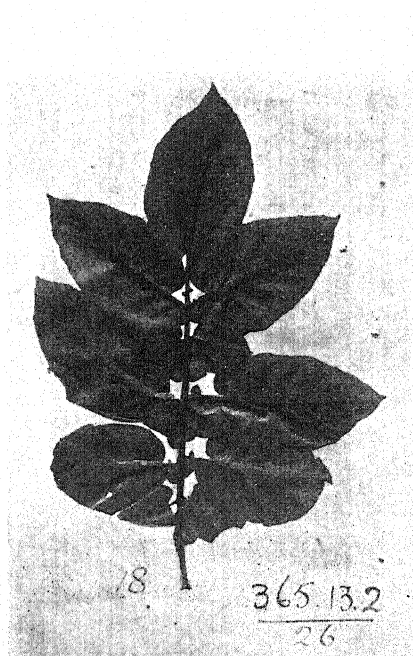
Text-fig. 15.
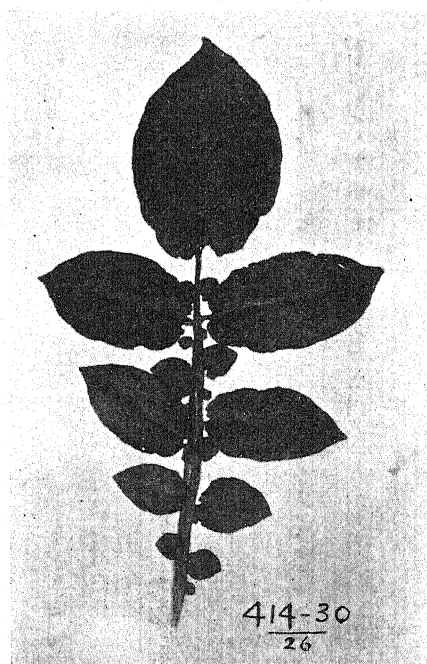
Text-fig. 16.

Text-fig. 15. Pressed leaf of domestic type of plant No. 18 of the family 365.13.2 which is otherwise uniformly of *S. utile* type.

Text-fig. 16. A leaf of 414.30—one of the members of the back-cross family which reproduced all the characters of the original F_1 family except for the absence of berries.

TABLE I.

Family 366.4.4.

No. of plant	Terminal leaflet		Distal end of leaflet		Apical process		Spacing of leaflets		Leaflets		Merging of leaflet and petiole		Folioles	
	Small	Large	Blunt	Sharp	Present	Absent	Narrow	Wide	Stalked	Sessile	Present	Absent	Small	Large
3	×	.	.	×	×	.	.	×	.	×	.	×	×	.
8	×	.	.	×	×	.	.	×	×	.	.	×	×	.
13	×	.	.	×	×	.	×	.	×	.	.	×	.	×
17	×	.	.	×	×	.	×	.	×	.	.	×	×	.
18	.	×	.	×	×	.	×	.	.	×	×	.	×	.
21	×	.	.	×	×	.	×	.	×	.	.	×	×	.
23	×	.	.	×	×	.	.	×	.	×	×	.	×	.
25	×	.	×	.	×	.	×	.	×	.	.	×	×	.
26	×	.	.	.	×	.	×	.	×	.	.	×	.	×
27	×	.	.	×	×	.	.	×	×	.	.	×	×	.
28	×	.	.	×	×	.	×	.	×	.	.	×	×	.
30	×	.	.	×	×	.	×	.	×	.	.	×	.	×
31	×	.	×	.	×	.	×	.	×	.	.	×	×	.
35	×	.	×	.	×	.	×	.	.	×	.	×	×	.
36	×	.	.	×	×	.	×	.	×	.	.	×	×	.
38	×	.	.	×	×	.	×	.	×	.	.	×	×	.
39	×	.	.	×	×	.	×	.	×	.	.	×	×	.
41	×	.	.	×	×	.	×	.	×	.	.	×	×	.
44	×	.	×	.	×	.	.	×	×	.	.	×	.	×
45	×	.	.	×	×	.	×	.	.	×	.	×	×	.
46	.	×	×	.	×	.	×	.	.	×	.	×	.	×
48	×	.	.	×	×	.	×	.	×	.	.	×	×	.
49	×	.	.	×	×	.	.	×	×	.	.	×	×	.
50	×	.	.	×	×	.	×	.	×	.	.	×	×	.
51	×	.	.	×	×	.	.	×	×	.	.	×	×	.
52	×	.	×	.	×	.	×	.	×	.	.	×	.	×
54	×	.	.	×	×	.	×	.	×	.	.	×	.	×
57	×	.	×	.	×	.	×	.	.	×	.	×	×	.
28	26	2	7	21	28	0	21	7	21	7	2	26	21	7

TABLE II.

Family 366.4.1½.

No. of plant	Terminal leaflet		Distal end of leaflet		Apical process		Spacing of leaflets		Leaflets		Merging of leaflet and petiole		Folioles	
	Small	Large	Blunt	Sharp	Present	Absent	Narrow	Wide	Stalked	Sessile	Present	Absent	Small	Large
5	×	.	.	×	×	.	×	.	.	×	.	×	×	.
6	×	.	×	.	×	.	×	.	.	×	.	×	×	.
8	.	×	×	.	×	.	×	.	.	×	.	×	×	.
13	.	×	×	.	×	.	.	×	.	×	×	.	×	.
14	×	.	.	×	×	.	×	.	.	×	×	.	×	.
16	×	.	.	×	×	.	×	.	.	×	×	.	×	.
18	×	.	.	×	×	.	×	.	.	×	×	.	×	.
19	×	.	×	.	×	.	×	.	.	×	.	×	×	.
21	.	×	.	×	×	.	×	.	.	×	.	×	×	.
23	×	.	.	×	×	.	.	×	.	×	×	.	×	.
29	.	×	.	×	×	.	×	.	.	×	.	×	×	.
30	×	.	.	×	×	.	.	×	.	×	.	×	×	.
37	×	.	.	×	×	.	×	.	.	×	.	×	×	.
40	×	.	.	×	×	.	.	×	.	×	.	×	×	.
44	×	.	.	×	×	.	×	.	.	×	.	×	×	.
15	11	4	4	11	15	0	11	4	0	15	5	10	15	0

TABLE III.

Family 417.

No. of plant	Terminal leaflet		Distal end of leaflet		Apical process		Spacing of leaflets		Leaflets		Merging of leaflet and petiole		Folioles	
	Small	Large	Blunt	Sharp	Present	Absent	Narrow	Wide	Stalked	Sessile	Present	Absent	Small	Large
1	×	.	×	.	×	.	×	.	×	.	.	×	×	.
2	×	.	.	×	×	.	×	.	×	.	.	×	×	.
3	×	.	.	×	×	.	×	.	.	×	.	×	×	.
5	×	.	.	×	×	.	×	.	×	.	.	×	.	×
6	×	.	.	×	×	.	.	×	×	.	.	×	.	×
9	×	.	.	×	×	.	×	.	×	.	.	×	.	×
11	×	.	.	×	×	.	×	.	.	×	.	×	×	.
12	×	.	.	×	×	.	×	.	×	.	.	×	×	.
13	×	.	×	.	×	.	×	.	×	.	.	×	.	×
15	×	.	.	×	×	.	×	.	×	.	.	×	.	×
17	×	.	×	.	×	.	.	×	×	.	.	×	×	.
18	×	.	.	×	×	.	×	.	×	.	×	.	×	.
19	×	.	.	×	×	.	×	.	×	.	.	×	.	×
20	×	.	.	×	×	.	×	.	×	.	.	×	×	.
21	×	.	.	×	×	.	×	.	×	.	.	×	.	×
22	×	.	.	×	×	.	×	.	×	.	.	×	×	.
23	×	.	.	×	×	.	×	.	×	.	×	.	.	×
24	×	.	.	×	×	.	.	×	×	.	.	×	×	.
27	×	.	.	×	×	.	×	.	×	.	.	×	×	.
28	×	.	.	×	×	.	×	.	×	.	.	×	×	.
29	×	.	.	×	×	.	×	.	×	.	.	×	.	×
30	×	.	.	×	×	.	×	.	×	.	.	×	.	×
34	×	.	.	×	×	.	×	.	×	.	.	×	.	×
39	×	.	×	.	×	.	×	.	×	.	.	×	.	×
43	×	.	.	×	×	.	×	.	×	.	.	×	×	.
45	×	.	.	×	×	.	×	.	×	.	.	×	.	×
47	×	.	.	×	×	.	.	×	×	.	.	×	×	.
49	×	.	.	×	×	.	×	.	×	.	.	×	×	.
50	×	.	.	×	×	.	×	.	×	.	.	×	.	×
51	×	.	×	.	×	.	.	×	×	.	.	×	.	×
52	×	.	.	×	×	.	×	.	×	.	.	×	.	×
53	×	.	.	×	×	.	×	.	×	.	.	×	×	.
55	×	.	.	×	×	.	.	×	×	.	.	×	×	.
56	×	.	×	.	×	.	×	.	×	.	.	×	.	×
59	×	.	.	×	×	.	×	.	×	.	.	×	.	×
60	×	.	.	×	×	.	×	.	×	.	.	×	×	.
61	×	.	.	×	×	.	×	.	×	.	.	×	.	×
62	×	.	.	×	×	.	×	.	.	×	.	×	×	.
38	38	0	6	32	38	0	32	6	34	4	2	36	19	19

5. The folioles are big, though not easy to see in the illustration, Text-fig. 4, as they are covered by the leaflets.

Pressed samples of both adult and young leaves from as many plants as possible were collected in 1926 from some of the F_3 and F_4 families, as well as the back-crosses, and these have since been examined with reference to these characters. The analyses, which rest essentially on the examination of the adult leaves, are found in Tables I, II and III. From these it is evident that the characters enumerated are, with certain reservations, simple ones, and that they segregate, again with certain reservations, with perfect regularity. Thus, the domestic terminal

leaflet type, *i.e.* "small," one not more than one-and-a-half times the area of the first lateral leaflet, behaves as a clean dominant over the large terminal of *S. utile*. The sharp point or apical process of the leaflet is not only dominant, but it is very doubtful whether in the subsequent generations the recessive rounded margin ever segregates out apart from all the *S. utile* specifically morphological characters; at least no example of such has been recorded.

Perhaps the type of leaf which is nearest to that of *S. utile* without being identical with it, is represented in Pl. VIII, fig. 1. In this leaf, two characteristics peculiar to the domestic type of leaf are still asserting themselves; the leaflets, though broad, have still a quite definite apical process, and the angle of insertion, though wide, is still less obtuse than is to be found in leaves of *S. utile*.

The characters "Blunt," "Sharp," must be carefully differentiated from the morphologically allied ones of the absence or presence of an apical process. Indeed, this latter, the presence of an apical process, seems to be a specific character of the domestic potato and never to be absent from a leaf which is not entirely *S. utile* in type. In the tables it will be seen to undergo no segregation.

"Blunt," "Sharp," on the other hand, are leaflet-shape characters which segregate and may be found in both wild and domestic types of leaf. The character "Blunt" or "Sharp" is determined by the shape of the distal end of the first lateral leaflet of a well-grown leaf. Both types of leaflet shape may be associated with a well-developed apical process. It will be observed that the inheritance of this character in this group of families may be abnormal. Thus, in the family 417 five individuals out of 28 show the broadened distal contour when none were expected; this may be due to a failure of dominance or, more probably, some chromosome irregularity.

The merging or fusion of the wing of the leaflet with the petiole of the stalk, if present, or its continuation with the petiole of the leaf, is not seen in domestic varieties, although a slight tendency in this direction has been observed in the case of the terminal leaflets of the varieties King Edward and President when grown under insect-proof cages. In *S. utile* itself the terminal leaflet almost invariably merges on one or both sides with the leaf petiole, so that the leaf terminates in a fusiform manner (see Text-figs. 1 and 2). The lateral leaflets, which are sessile in *S. utile*, only very rarely merge with the petiole of the leaf, and then over a short distance only. In the F_1 plants there may be a slight merging of the terminal leaflet but there is none of the laterals. The
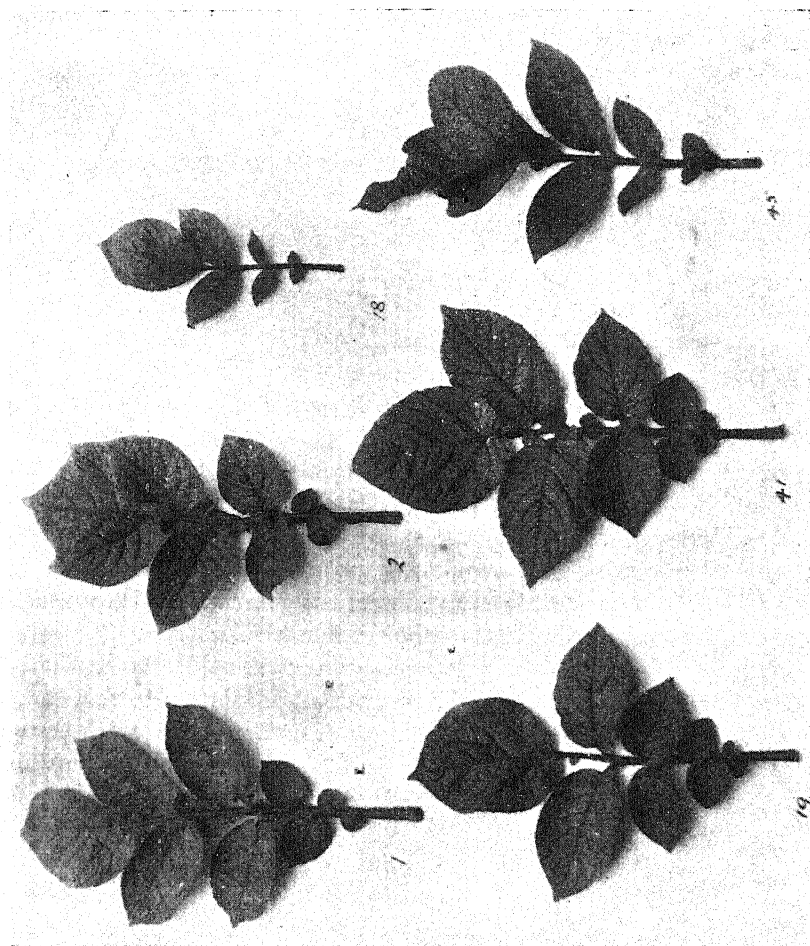
Text-fig. 17. Leaves from six plants of the family 417.17 showing varying degrees of merging of the lamella of the leaflet with the petiole.

character was only studied in one of the non-*utile*-like F_2, F_3 and F_4 families of the 365 group, viz. 365.18.18. In this family 9 leaves were preserved and merging is present in 1 plant. It was in the 366 group that a more exact study of this feature was possible. Here, as in the F_1 family 365, the F_1 family 366 shows no merging except an occasional very slight extension of one or other side of the terminal leaflet into the petiole. In the F_3 family 366.4.4 fusion of the terminal leaflet and the petiole was seen in one plant, and a similar fusion of the terminal as well as of the first lateral leaflets, in another; two cases out of 28 in which the leaves had been preserved. In the sister family, 366.4.11, out of 15 plants of which the leaves had been preserved, in 5 merging was present in both the terminal and first lateral leaflets; and in the back-cross family 417 (see Pl. VIII, fig. 6) 2 out of 38 plants were similarly affected.

In the family 417.17 derived from the last, the feature was studied in the growing plants (Text-fig. 17) and in several plants this merging of the terminal and first lateral leaflets into the petiole has developed into a very striking feature. Segregation of this character was quite definite, viz. 16 with merging, and 44 without. Folsom(1) has described a leaf mutation in the variety White Mountain which is very similar to the merged or adnate condition of the lamella of the leaflets seen in 417.17.1, Text-fig. 17; it persisted through four tuber-generations.

The tendency to merging would thus appear to have been introduced by *S. utile* and to be recessive. In subsequent generations it reappears in the families which are not morphologically similar to *S. utile* in a much intensified form, affecting not merely the terminal leaflet but both that and the first laterals to a degree out of all proportion to that seen in the *S. utile* parent. Its behaviour in the four families in which there was material to examine it, suggests that the gene originally derived from *S. utile* behaves as a simple recessive, but that in the presence of some of the chromosomes of the domestic parent the expression of the character is greatly intensified. The presence of two only individuals, with merged leaflets, in families of 38 and 28 individuals respectively, where all the remainder are unmerged, is probably to be ascribed to the irregular distribution of the chromosomes which, as will be shown in a later paper, is so common in these specific hybrids.

Spacing of the leaflets on the main axis is described under the terms "narrow" and "wide"; by "narrow" is meant when the space between the insertions is less than one-half the length of the leaflet plus whatever pedicel it may have, and by "wide" when it is more than one-half that length. This character is a perfectly sound one and segregation occurs

in both families, 366.4.4 and 366.4.11, in a straightforward 3 : 1 manner. In the back-cross family 417 it was to be expected that all the 38 individuals examined would be "narrow" but, in fact, 6 were classed as "wide." The leaflets of 5 of the 6 are unquestionably widely spaced, the sixth is, however, peculiar inasmuch as one fair-sized leaf is "narrow" and a bigger leaf falls just into the "wide" class.

A stalked condition of the leaflet is a stable character of the domestic potato and is dominant to the sessile or quasi-sessile, a character of *S. utile*. With regard to this character we find that in family 366.4.11, where the plants on the whole tend to assume a predominatingly domestic type, all the 15 individuals are pure for the *S. utile* feature "sessile." In family 366.4.4 there is a normal 3 : 1 segregation. In the back-cross family 417, of 38 individuals of which leaves were preserved, four "sessiles" appear, when none were expected.

The presence and absence of folioles is not the clean-cut character it might have been thought to be, and the *S. utile* leaves may either have none, or folioles of minute size may develop, and so in the subsequently derived *S. utile*-like families one leaf may be totally devoid of folioles, another may have a few minute ones. Here an attempt has been made to distinguish between large folioles and small: a small foliole has been defined as one not more than one-fifteenth the size of the first lateral leaflet. In practice, there is no real difficulty in distinguishing the "small" from the "large"; moreover, with the "large" goes generally a multiplicity of folioles; with "small," a sparseness of the same. Small foliole, a *S. utile* character, is dominant. In 366.4.11 the family is purely dominant for this character, whilst in 366.4.4 there is a perfect 3 : 1 segregation, and in the back-cross family there is, as was to be expected, a 1 : 1 result. Small (and few) folioles is the only leaf character of *S. utile* which is dominant.

Amongst the distinguishing leaf characters noticed earlier in the paper was the angle of insertion of the leaflet into the main axis of the leaf. This feature has not been further elaborated in the analysis made from the dried specimens because the method of fixing the leaves was inclined to distort this relation. It can, however, be stated positively that the insertion of a leaflet at an angle of 60° or thereabouts, which is characteristic of the domestic leaflet, is never lost except in those cases where the *S. utile* plant as a whole is reproduced. Indeed, this leaf character reacts hereditarily in the same manner as does the acute apical process of the leaflet.

Having analysed the leaf characters, it is interesting to observe in

the tables their independence of one another. The result of such independence, coupled with the peculiar nature of the inheritance of the "apical process" and "angle of insertion," is that the *S. utile* leaf can only be reproduced when the entire plant is reformed. The domestic leaf, on the other hand, can be and is reproduced when other domestic characters such as those of flower and haulm are not present in the plant, but owing to the independence of the characters this is not of very frequent occurrence. In family 366.4.4 the domestic type of leaf in its purity appears 4 times out of 28. In family 366.4.11 it is not reproduced at all amongst 15 individuals; and in the back-cross family 417, it occurs 14 times out of 38.

Flower colour. The domestic parents used were all white flowered. The *S. utile* flower is of a deep bluish-purple. Pigment, when present in the domestic varieties, is deposited in the upper layer of the mesophyll of the corolla, whilst in the immediate region of the bundles some may occur in the lower layers as well. In some white-flowered varieties, *e.g.* Flourball, a pure blue pigment is found in the lower mesophyll covering the vascular bundle.

In the F_1 families 365 and 366, as well as the two back-cross families 414 and 416 which so closely resembled them, the corolla is uniformly blue, and the colour is deposited mainly in the lower surface, a little being present in the upper.

Where segregation of haulm characters occurred, in the families arising from the two original crosses, *i.e.* in those families of F_2, F_3, etc., generations which were not entirely dominated by *S. utile*, segregation in respect to both colour and locale of the pigment was observed.

TABLE IV.

No. of family	Generation	Red surfaces			Blue surfaces		
		Upper	Under	Both	Upper	Under	Both
365.18.	F_2	0	0	0	0	12	3
365.18.18.	F_3	0	0	2	0	10	1
366.4.	F_2	1	0	0	0	7	4
366.4.4.	F_3	1	5	2	4	3	8
366.4.11.	F_3	1	2	0	0	7	0
366.27.	F_2	0	0	0	0	1	5
366.46.	F_2	0	0	0	0	0	4
415.	$F_2 \times P$ (domestic)	0	0	0	0	4	2
417.	$F_3 \times P$ (domestic)	3	4	1	0	19	2
417.17.	F_2	0	0	0	0	40	0
417.50.	F_2	0	0	0	0	5	0
		6	11	5	4	108	29

Total of red flowers 22: of which colour is present in upper surface only in 27 per cent.
Total of blue flowers 141: of which colour is present in upper surface only in 2·8 per cent.

In Table IV it will be seen that the segregation as regards surface distribution is not a simple one. Under-surface pigmentation is clearly dominant though the heterozygote may show some pigmentation in the upper surface as well. There is obviously a close linkage between the factor for blue pigmentation, which is dominant to red, and that for under-surface distribution. Red, *i.e.* pale heliotrope flowers are devoid of the blue factor brought in by *S. utile,* and there is a linkage between the red factor and that for upper-surface distribution.

CROPPING CAPACITY.

The inheritance of cropping, as estimated by the author's method(8) of cropping curves, has been studied on these *S. utile* and domestic crosses with some success. *S. utile,* itself a zero cropper, produces a crop of curve Type VII[1] (see Text-fig. 19). The domestic parent's crop curve is of Type III (see Text-fig. 19).
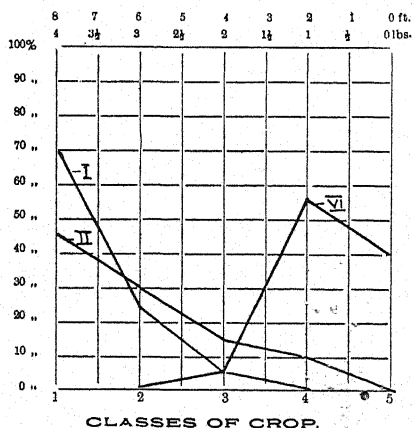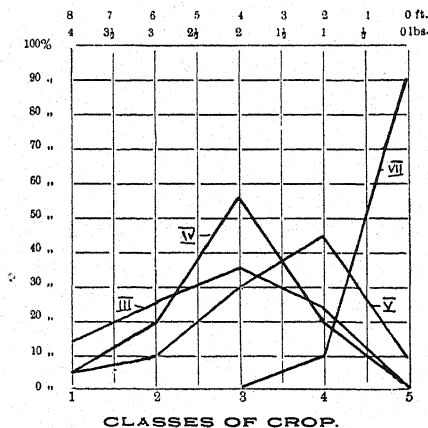
Text-fig. 18.   Text-fig. 19.

Text-figs. 18, 19. Diagrams representing the seven types of crop curves.

The F_1 in all five cases where it has been crossed by domestics, produces a perfectly uniform cropping curve of Type VI. From the earlier crosses not further specifically described in this paper, four F_2

[1] It should be explained that for the purpose of these curves, the individuals of a family are divided into five classes in respect of their yields:

1. Those whose yield is very good in relation to size of haulm.
2.   ,,   ,,   fair   ,,   ,,
3.   ,,   ,,   poor   ,,   ,,
4.   ,,   ,,   extremely small in relation to size of haulm.
5.   ,,   ,,   nil.

families were raised. They were, however, so small, viz. from 6 to 12 plants in each, that they did not afford sufficient evidence to allow of any general deductions. Two facts, however, were apparent: the one that, if a plant which was itself a "cropper" and derived from, or itself giving rise to, a family curve of Types I to IV was mated with a "zero" cropper, the latter was dominant, *i.e.* it resulted in the more or less complete inhibition of cropping in the F_1 plants. The power of cropping, however, is not lost as was proved by some of the F_2 families which, small as they were, included several individual plants amongst them which produced quite good crops. The second point is that in those four F_2 families, in which a more or less free segregation of morphological characters occurred, the family cropping curve Type VII peculiar to *S. utile* did not or would not have recurred, however big the families had been. This statement implies a linkage between the genes responsible for a cropping capacity as represented by Curve VII (and possibly Curve VI), and those genes which control the production of the *S. utile* characters of foliage and habit. That this is indeed so, is confirmed by the fact that all the 13 *S. utile*-like families that arose in the F_2, F_3 and F_4 families of the 365 group were possessed of curves whose type was VII.

In the two interspecific crosses of *S. utile* by domestic varieties dealt with in this paper, we have seen the occurrence of a "mass" somatic segregation, and a "mass" genetic segregation of characters common to one parent, occurring in the same individuals, and at the same time the retention in a recessive or latent condition of other characters derived from the other parent, and it was of interest to discover whether anything on the same scale was taking place in regard to cropping.

The evidence is clear. Every one of the F_2, F_3 and F_4 families which reproduced the *S. utile* characters in their entirety, reproduced the corresponding No. VII type of cropping curve. When, however, the back-crosses were studied, it was clear that however similar these families were to *S. utile* in appearance, there were hidden differences. Thus the two back-cross families 414 and 416 (see Text-fig. 6) reproduced to all appearances the original F_1 type, except for the berries, but in place of the F_1 cropping curve they reproduced a modified No. IV curve, both families containing roughly 30 per cent. of individuals giving a No. 3 crop, whereas no F_1 plant bore more than a No. 4 crop, *i.e.* a very poor crop. Still more interesting was the result of the back-cross 415, for here one parent was an F_2 from the original mutating F_1. The family produced was rather small, the cropping curve was of Type IV, showing nearly 10 per cent. of "good" crop and 10 per cent. of "fair" crop,

which is strong evidence that although one parent visibly resembled *S. utile*, it had retained certain genes for cropping derived from the original domestic parent.

In the progeny derived from the F_1 plants 365.18 (Text-fig. 6), as well as those of the 366 group (Text-fig. 7), in both of which segregation of the morphological characters occurred more or less freely, a few only of the families so far harvested are large enough to be of value. There are, however, five families whose populations were 14, 17, 20, 25 and 32 respectively, and these display cropping curves which may be referred to Types III, VI, V, VII and VII, indicating a definite segregation of crop-controlling genes and a confirmation of the view that inhibitory factors are dominant.

Conclusions.

1. Crosses between *S. utile* and domestic potatoes have only taken place when *S. utile* acted as the maternal parent.

2. Whatever the domestic parent used, the F_1 family has been the same in all its external characters and, with the three exceptions noted in the text, the component individuals are identical.

3. The crosses of *S. utile* × domestic potato have been traced through five generations, and certain back-crosses have been made.

4. The outstanding feature of the whole series has been the occurrence of families in the F_2, F_3 and F_4 generations pure in type and indistinguishable from the *S. utile* parent. This phenomenon is described as "mass segregation."

5. Evidence is adduced to show that although these families are in their morphological characters indistinguishable from *S. utile*, there are genetic and physiological differences which are demonstrated by the result of back-crosses, the presence of sterility and the incidence of disease.

6. No family pure to the domestic type has been recovered, although others occur where all the seedlings are "nearly," and some quite, domestic in character. The reason of the non-appearance of the pure domestic family may be due to the free segregation of the characters, especially those of the leaf. In two of these families a single individual has occurred with all the morphological characters of *S. utile* perfectly reproduced.

7. Differential morphological characters of the leaves of *S. utile* and the domestic potato are described, and their inheritance discussed.

8. Two features of the domestic leaflet are never lost in succeeding

generations except when all the *S. utile* plant characters reappear *en masse,* viz. the sharp apical prominences to the leaflet and the angle of leaflet insertion which is always below 80°.

9. The recessive character of "merging" derived from *S. utile* is greatly intensified in its expression, though still remaining recessive, in the presence of chromosomes bearing "domestic" characters.

10. The blue flower pigment of *S. utile* is dominant to the white and red of the domestic varieties. The factor determining deposition of pigment in the lower layers of the mesophyll of the corolla is dominant to that determining deposition in the upper. There is a close linkage between the factor for blue pigment and that for lower layer deposition, and also between that for red pigment apart from blue, and deposition in the upper layer of the mesophyll.

11. The cropping capacity, as measured by cropping curves of the families from *S. utile* by domestic crosses, demonstrates that the *S. utile*-like families of F_2, F_3 and F_4, whilst producing crop curves precisely the same as those of *S. utile,* are nevertheless carrying genes for higher cropping in a latent condition. There is clear evidence of segregation of cropping factors in the non-*utile*-like families, and in all families the inhibitory factors derived from *S. utile* are dominant.

12. In an inter-species cross, *S. utile* × *S. chacoense,* both the F_1 and F_2 are completely of the *S. utile* type.

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EXPLANATION OF PLATES V—VIII.

PLATE V.

Fig. 1. A plant of *S. utile.*

Fig. 2. The domestic parent 113.18.1.

PLATE VI.

The plant 365.13 photographed in 1924 after it had acquired the normal F_1 appearance.

PLATE V

Fig. 1.

Fig. 2.
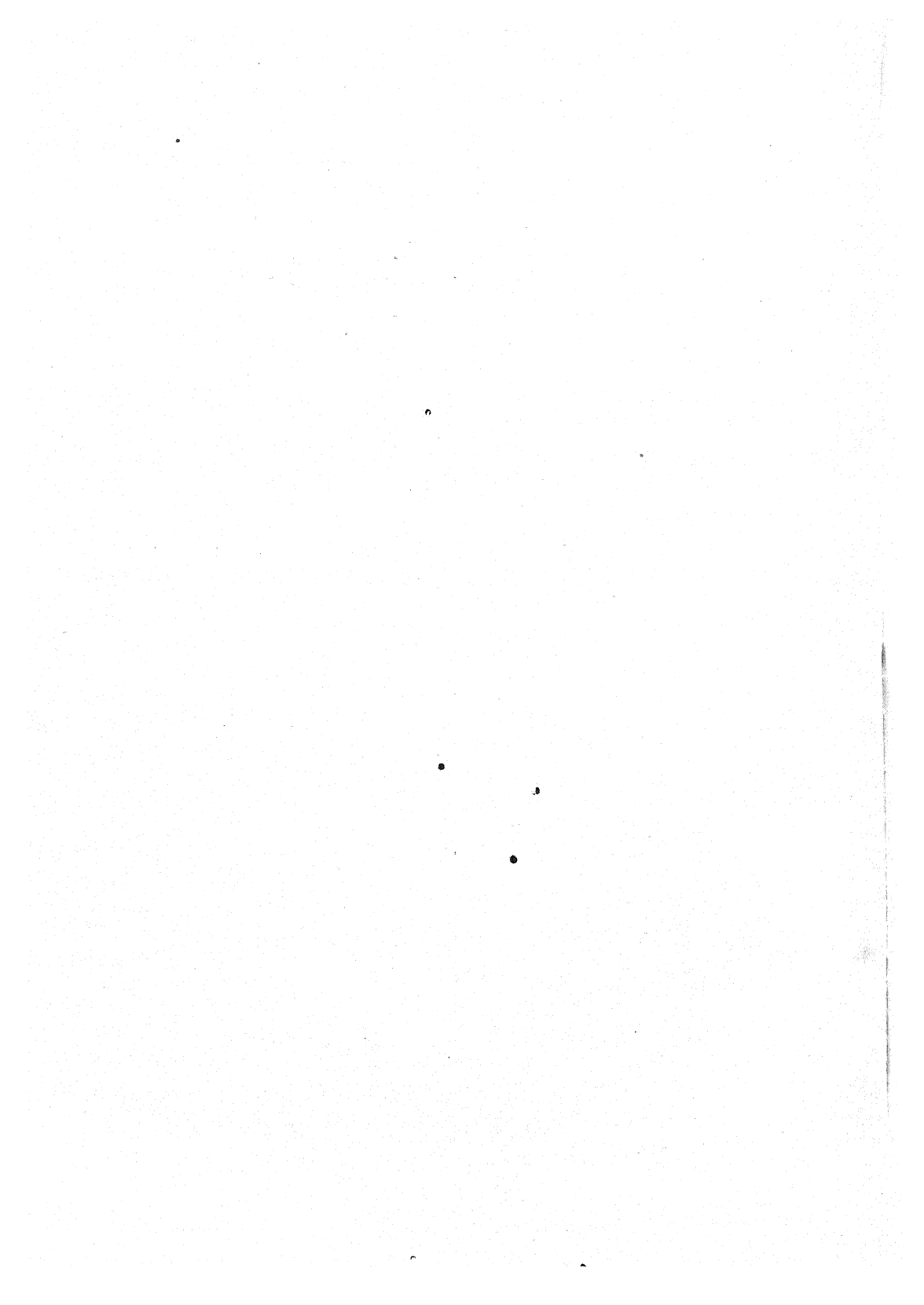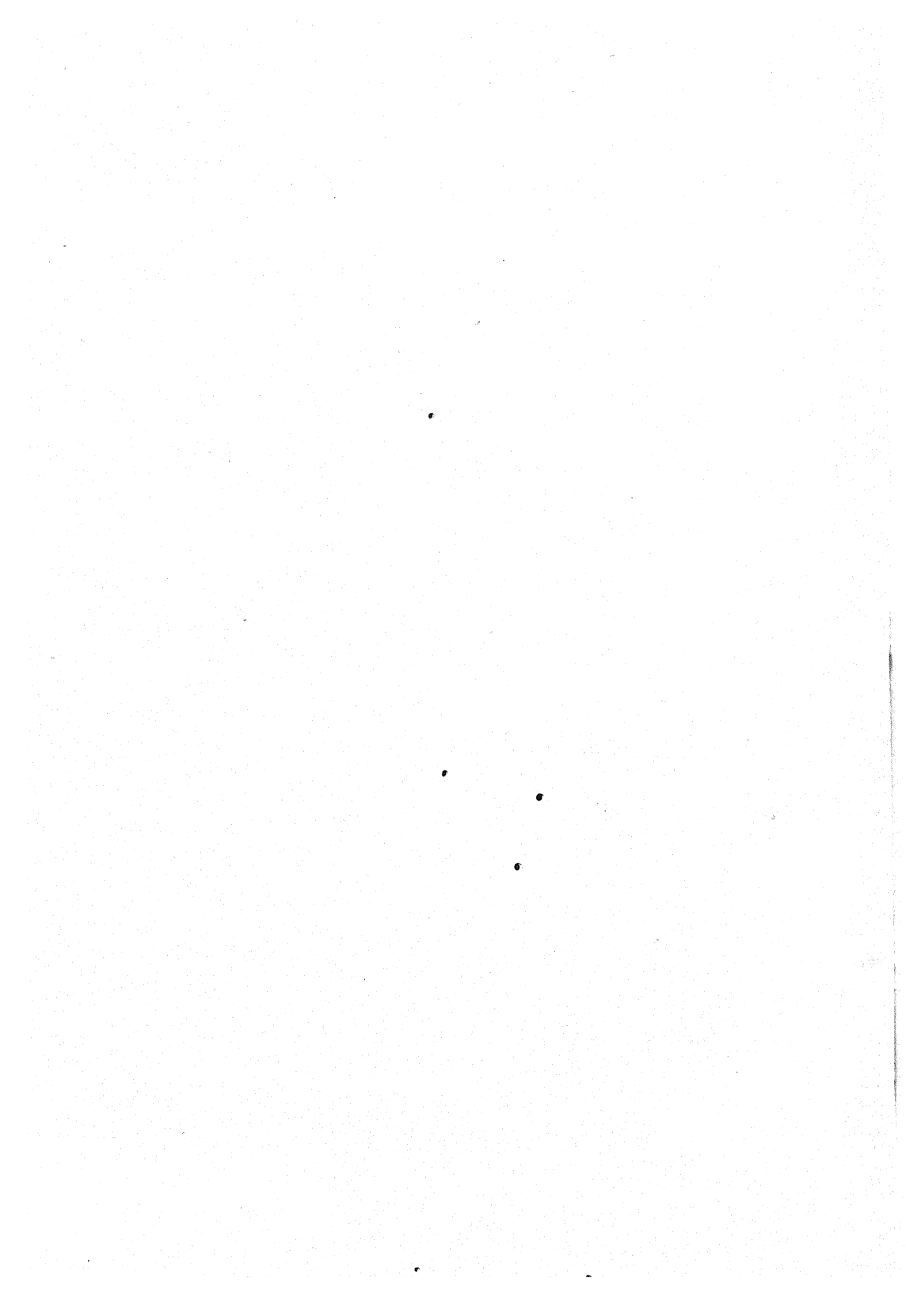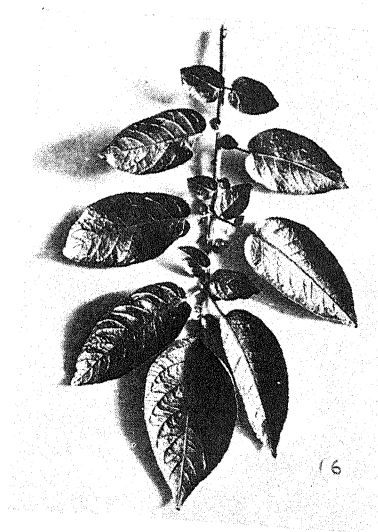
PLATE VI
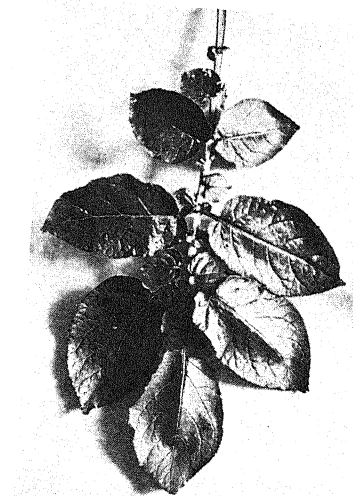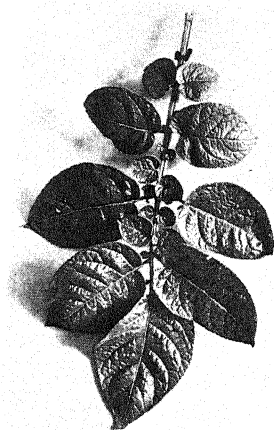
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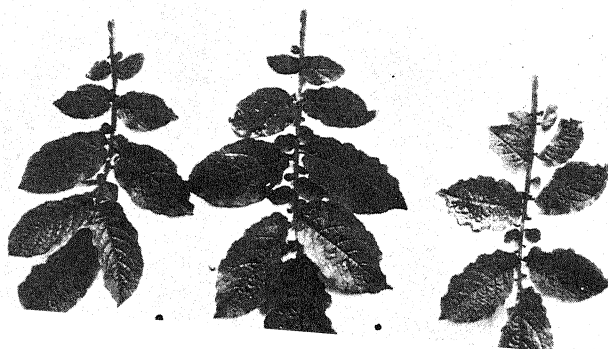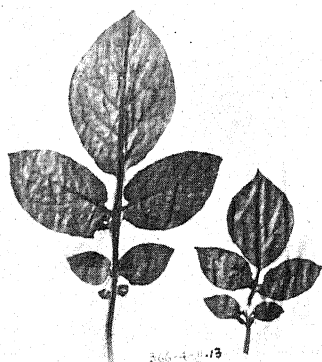
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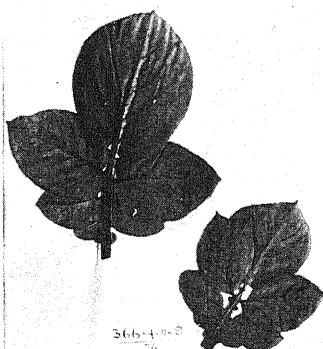
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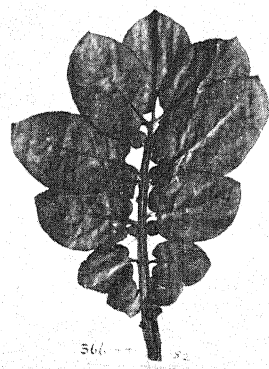
Fig 3.

Fig. 4.

Fig. 1.

Fig. 2.

Fig. 3.
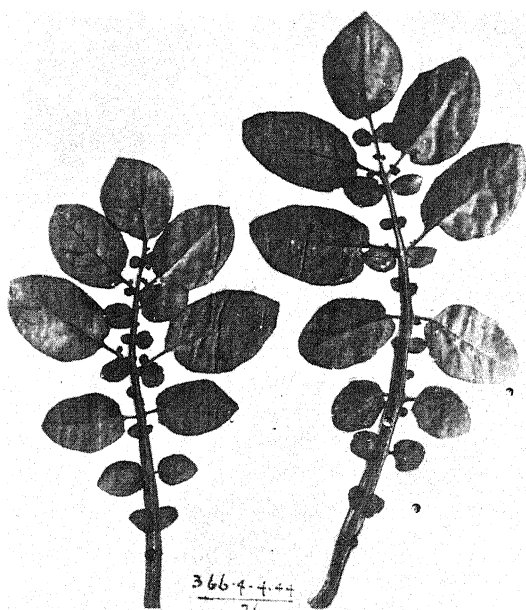
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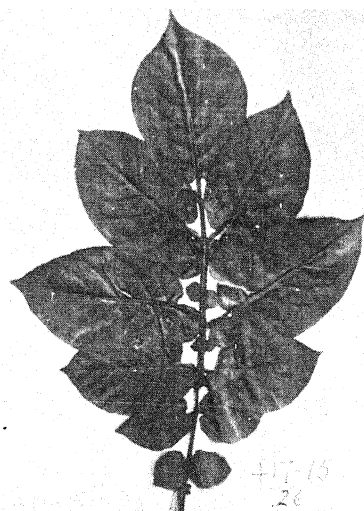
Fig. 6.
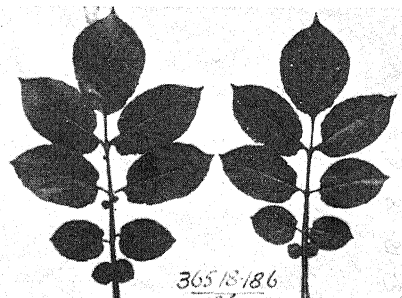
Plate VII.

Fig. 1. Leaf of the variety Mr Bresee.

Fig. 2. Leaf of the variety Great Scot.

Fig. 3. Leaf of the variety Leinster Wonder.

Fig. 4. Leaf of the variety Sharpe's Express.

Figs. 5, 6, 7. Leaves of the F_1 plants 365.13, 365.10, 365.18. All as regrown in 1924. It is to be noted that the leaves of plants Nos. 10 and 13 are now exactly similar to the normal F_1, No. 18.

Plate VIII.

Figs. 1–6 show several of the combinations of leaf characters which occur in the F_4 families and back-crosses. At the beginning of the series is a leaf, Fig. 1, which would be a perfect *S. utile* type were it not that the angle of insertion is too small and an apical process is present; whilst in Fig. 6, the domestic type is reproduced in its entirety. In between these extremes, occur all sorts of bizarre character groupings which may give rise to monstrosities such as is represented in Fig. 4.

	Domestic characters	*S. utile* characters
Fig. 1.	Apical process	—
	—	Large terminal leaflet
	—	Blunt leaflet
	—	Wide spacing
	—	Sessile leaflets
	—	Folioles few and small
	Angle of insertion less than 80°	—
Fig. 2.	Apical process	—
	—	Large terminal leaflet
	—	Blunt leaflet
	Narrow spacing	—
	—	Sessile leaflets
	—	Folioles few and small
	Angle of insertion less than 80°	—
Fig. 3.	Apical process	—
	Small terminal leaflet	—
	—	Blunt leaflet
	Narrow spacing	—
	Stalked leaflets	—
	Large folioles	—
	Small angle of insertion	—
Fig. 4.	Apical process	—
	Small terminal leaflet	—
	—	Blunt leaflet
	—	Wide spacing
	Stalked leaflets	—
	Large and numerous folioles	—
	Small angle of insertion	—
Fig. 5.	Apical process	—
	Small terminal leaflet	—
	Pointed leaflet	—
	Narrow spacing	—
	—	Small and few folioles
	Stalked leaflet	—
	Angle of insertion less than 80°	—
Fig. 6.	Apical process	—
	Small terminal leaflet	—
	Pointed leaflet	—
	Narrow spacing	—
	Stalked leaflet	—
	Large and numerous folioles	—
	Angle of insertion less than 80°	—
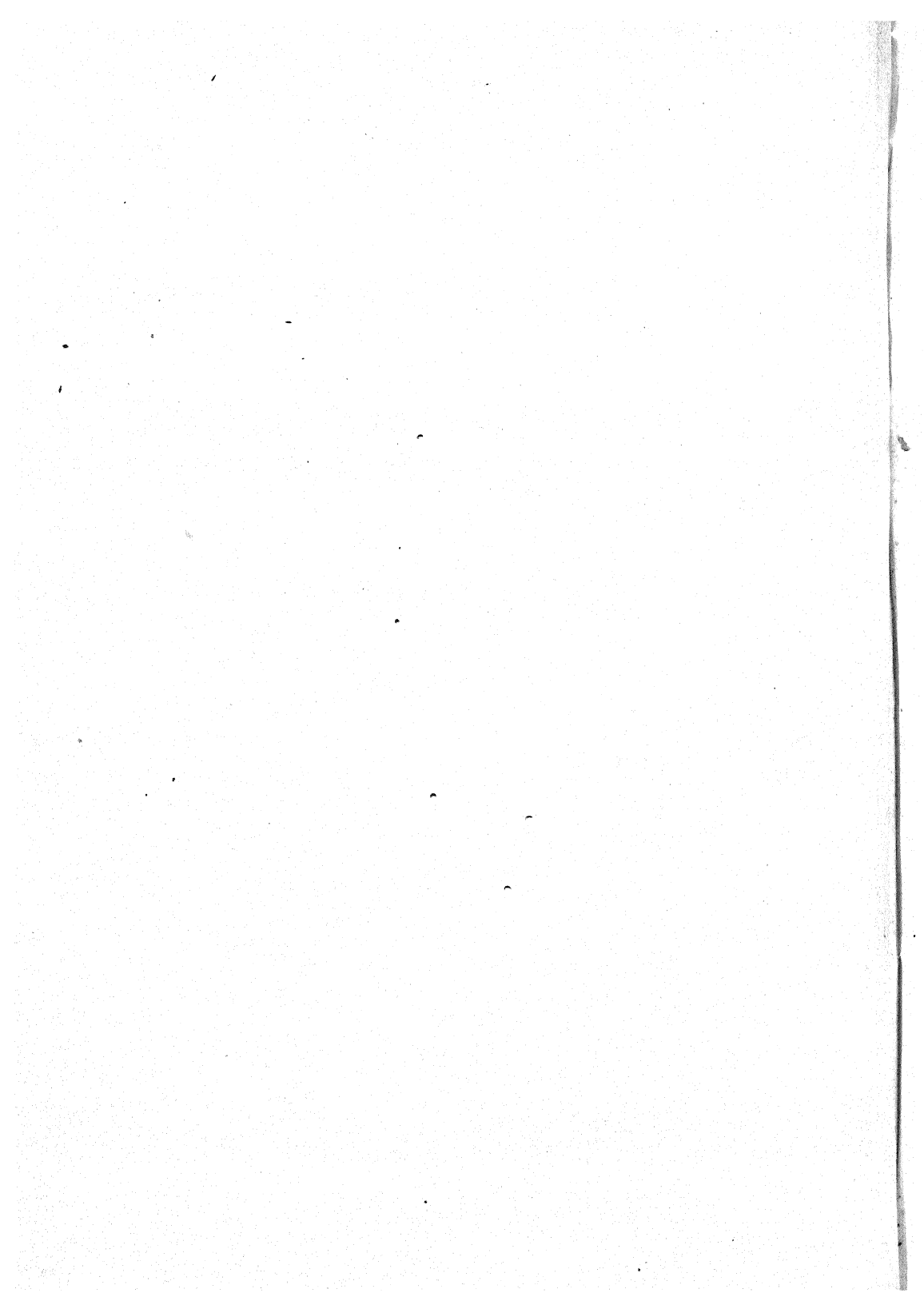
RING-FORMATION IN *OENOTHERA* AND OTHER GENERA.

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(With One Diagram.)

EVER since ring-formation was found in *Oenothera rubrinervis* (Gates, 1908) this behaviour of the chromosomes has offered a twofold problem: first, its correlation with the genetical behaviour of the plant; secondly, its correlation with the behaviour of the chromosomes at meiosis in other plants and animals.

In regard to the first part of the problem we now know, thanks to the work of Oehlkers, Cleland and others, that a chromosome ring in *Oenothera* corresponds to two sharply defined linkage groups, Renner's complexes. It has been assumed that, as alternate members of the ring go to the same pole, successive chromosomes must be maternal and paternal. Thus, although the method is exceptional, we must admit that in the genetical linkage of factors associated with a corresponding linkage of chromatin elements at reduction *Oenothera* shows an essential similarity with other organisms.

But further, in regard to the second part of the problem, diploid *Oenothera* has shown by the production of various kinds of trisomic mutants the same genetical differentiation within the chromosome complement that has now been established in such a great number of plants and animals. The seven chromosomes of the regularly formed gamete in *Oenothera* must therefore be complementary and not in any sense homologous to one another. In many other diploid organisms where *n* pairs of chromosomes are formed at the reduction division there is no difficulty in seeing that each of *n* types of chromosome is represented twice in somatic divisions; at meiosis therefore the *n* bivalent chromosomes are to be regarded as so many homologous pairs whose members separate to opposite poles. In the ring-forming *Oenothera* however each chromosome is associated with two others at the reduction division, and it *regularly passes to the opposite pole from both of these two.* In another important respect therefore, in spite of its anomalous behaviour, it fulfils the general conditions of meiosis. Its behaviour is indeed analogous with that of the *Phragmotobia* cross reported by Seiler (1925), where two small

chromosomes of one race pair with, and afterwards usually pass to, the opposite pole from one large one of the other race, with which they are presumed to be homologous.

What is the physical basis of the conditions of pairing in *Oenothera*? Work on *Tulipa* and *Hyacinthus* (Newton and Darlington) has shown that chromosome pairing at metaphase in these plants is determined by earlier conjugation between homologous *parts* of chromosomes, and not between the chromosomes as entities. Further, in polyploid tulips and hyacinths pairing at metaphase is shown to be maintained by the chiasmata formed at prophase, which may often be terminal and give the appearance of "end-to-end" pairing.

It is important to determine whether these principles, either as they stand or with some minor modification, can be applied to the interpretation of *Oenothera*. It will at once be objected that side-by-side conjugation, the essential feature of meiosis in other related and unrelated forms, is not generally admitted in *Oenothera*, nor does the behaviour of the chromosomes at diakinesis indicate any such earlier association. Boedijn (1924) however has described parasynapsis in diploid and tetraploid varieties[1]. We know that this system of pairing is subject to important variations both in method and result. In *Mecostethus* (Janssens, 1924) for example conjugation side by side takes place at one end only of the homologous chromosomes. In *Fritillaria* (Newton, 1927) a still more interesting variation occurs, for there apparently the point of attachment of the two chromosomes, Janssens' chiasma, is fixed at metaphase by the position of the attachment constriction. The occurrence of these modifications suggests that the parasynaptic system is sufficiently elastic to give results at metaphase of the most widely divergent appearance[2]. Moreover, the probability of the method of pairing in *Oenothera* being essentially parasynaptic has been urged upon specific grounds, genetical and cytological, by other workers (Oehlkers, 1924; Renner, 1925)[3]. There is thus no objection in principle to the view that ring-formation can arise from parasynapsis whether in a diploid or in a polyploid.

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[1] Quoted by Håkansson, 1927.

[2] This question will be dealt with in detail in a later paper.

[3] Renner's remarks are peculiarly appropriate to this discussion: "Mir scheint die optisch wahrnehmbaren Vorgänge an den Chromosomen irrelevant; wahrscheinlich spielt sich der Austausch von Chromosomenstücken in einem Zustand ab, in dem die Chromosomen noch nicht färbbar und nicht sichtbar sind und in so frühen Zuständen könnten die homologen Chromosomen ja auch bei *Oenothera* parallel gelagert sein." One amendment might be suggested: the difficulty has been in fixation rather than in staining at the zygotene stage.

In other genera, such as *Tradescantia*, ring-formation is the result of earlier side-by-side conjugation and association by terminal chiasmata (see note, p. 357). Similarly in *Rumex acetosella* Kihara has shown that ring-formation follows side-by-side conjugation. Recent work on the polyploid hyacinths (Darlington, in the press) as well as the observation of strings and rings of chromosomes in polyploid *Avena*, *Prunus*, *Primula*, *Solanum* and other genera make it very probable that ring formation in *Rumex* is actually the result of the side-by-side conjugation at different points of more than two homologous chromosomes, and that this species is in fact a polyploid. Moreover, Kihara's illustrations of *metaphase* show the preparation for a distribution of chromosomes characteristic of a polyploid (compare diagram, p. 348); they are incompatible with a diploid interpretation for adjoining chromosomes evidently pass to the same pole, and the characteristic arrangement of *Oenothera* is wanting.

Two differences can be observed between the condition in *Oenothera* and the one prevailing in autopolyploids. First, the pairing in *Oenothera* is evidently limited so that two chromosomes can pair at only one end, in those parts where they are presumably homologous, whereas in a polyploid, although pairing can take place at any one point between two only, it is free to take place between any two (Newton and Darlington, in the press). Secondly, at metaphase of the first maturation division separation in a polyploid normally takes place at random; in *Oenothera* paired chromosomes regularly pass to opposite poles. If then *Oenothera* is comparable with the rest of observed material in its method of pairing at prophase we cannot admit that the seven chromosomes of one complex are homologous with the seven of the opposite complex each to each; we must assume rather that they are homologous as a whole, but that each chromosome of one complex corresponds to parts of two chromosomes in the opposite complex; for each chromosome must be supposed to have paired with parts of two other chromosomes. Further, we must assume that the chiasmata at diakinesis are fixed and approximately terminal. It will be seen at once that from the observational point of view this might be called "telosynapsis"; but even this superficial view it is telosynapsis with a very special qualification, namely that only *homologous* parts of chromosomes can be supposed to be associated as a result of their earlier, and actually parasynaptic, conjugation.

The diagram on p. 348 illustrates the apparent similarity and the essential difference between a polyploid and diploid *Oenothera* as we must imagine it to be, on a parasynaptic basis. Corresponding
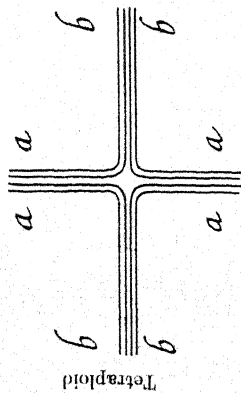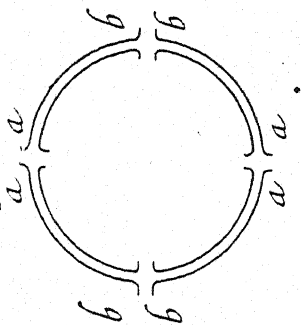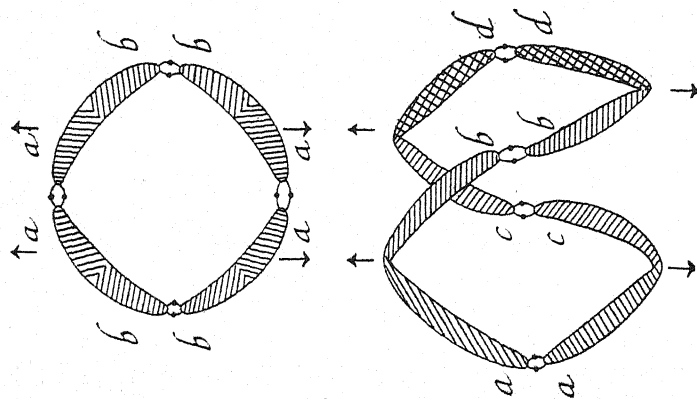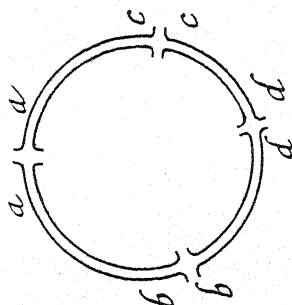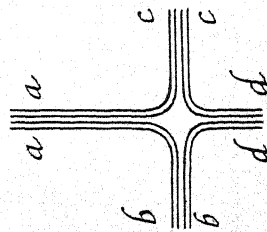
Diagram showing two possible types of ring formation

segments are paired at pachytene and associated by terminal chiasmata at diakinesis and metaphase.

In the tetraploid four homologous chromosomes are represented, each composed of two segments a and b. In the diploid four chromosomes are represented of the segmental constitution ab, bd, dc and ca thus corresponding genetically to the two pairs ab, ab, and dc, dc.

On the basis of the parasynapsis of homologous parts of chromosomes we have a problem of an entirely different character from that which has hitherto been envisaged. It perhaps appears more complex but its requirements can be stated with precision and do not admit of the elasticity in application with which the telosynaptic conception appears to be endowed.

We have to consider how the condition can have arisen in a diploid of a chromosome pairing along part of its length with one of the opposite set and along another part with a different one of that set; in other words, of being homologous in different parts with parts of different chromosomes.

There are in *Oenothera* forms that regularly have paired chromosomes, and since this, the ordinary condition in meiosis, is found in the related genera *Fuchsia*, *Godetia* and *Epilobium* it is easiest to suppose that simple pairing is the earlier condition in *Oenothera*. From the condition of simple pairing there are two ways in which chromosomes each capable of pairing with two others could arise; one is by fusion of non-homologous chromosomes to form, as it were, the "multiple chromosomes" of MacClung. A ring of six in *Oenothera* would thus be composed by the arrangement of six pairs of chromosomes A–F in the following fashion:

$$AB \qquad CD \qquad EF$$
$$BC \qquad DE \qquad FA$$

The other is by exchange of parts between non-homologous chromosomes. Exchanges between all the seven chromosomes making up one haploid set would be necessary to produce the ring of 14 chromosomes.

The former of these two ways, for various reasons which we need not go into now, seems too artificial to be considered. The latter on the contrary demands the continuation of a process of which the initial steps have been actually observed or inferred in *Datura* and *Drosophila*.

In *Datura* Blakeslee (1927) has described how forms attributed on genetical grounds to "segmental interchange" were found to produce rings of dissimilar chromosomes segregating so that not merely homologous chromosomes but parts of homologous chromosomes passed to opposite

poles, in the way required by the genetical behaviour of *Oenothera* and, on the hypothesis we are now considering, by its cytological behaviour. The diagram constructed by Belling illustrates exactly the process that I have in mind (*v.* Blakeslee, 1927).

Accidents of "translocation" and "sectional duplication" have been shown by genetic experiment in *Drosophila*. In their résumé of *Drosophila* work, Morgan, Bridges and Sturtevant observe that "the frequency of sectional-duplications is now supposed to be fairly high." Evidently a translocated section is usually attached to the *end* of the same chromosome or of another. Such a translocation, which is half the process of the "segmental interchange" with which we are concerned, would not necessarily affect pairing at meiosis. Where complete parasynapsis occurs and association is maintained at metaphase at points along the whole length of the chromosomes—as is probably the case in *Drosophila*—a small portion would not be likely to govern the behaviour of the rest. But in *Oenothera* where association is maintained only terminally at metaphase —whatever the earlier behaviour—and where the frequent absence of crossing over in the rings indicates incomplete synapsis, it is just the constitution of the end of the chromosome that would govern the metaphase association of the whole. And we may recall the fact that the behaviour of the parts of a chromosome as shown by Seiler's work is not affected by fragmentation, either in pairing or as a rule in segregation.

Finally, observations of fragmentation are a strong hint of the possibility of translocation or segmental interchange in both *Oenothera* and *Tradescantia* (see p. 358).

To describe now the hypothetical exchange in *Oenothera* it will be easiest to refer to each chromosome as though it consisted of two parts, as shown in the diagram. Thus the original condition would be, shall we say:

$$AB \quad CD \quad EF \quad GH \quad KL \quad MN \quad OP$$
$$AB \quad CD \quad EF \quad GH \quad KL \quad MN \quad OP$$

The condition of the 14-chromosome ring, the final product of interchange, would be:

$$AB \quad CD \quad EF \quad GH \quad KL \quad MN \quad OP$$
$$BC \quad DE \quad FG \quad HK \quad LM \quad NO \quad PA$$

The six necessary exchanges would have to be such that the distal ends of the old chromosomes remain the distal ends of the new—in order to retain the terminal attachment at metaphase. Thus our diagram would

show the first step in which *ab–ab* and *dc–dc* have become *ab*, *bd*, *dc* and *ca* by interchange of segments, *ab* and *dc* giving *bd* and *ca*.

It may be said that the completion of this process demands a series of coincidences of an exponential order of rarity; that it is therefore a highly artificial hypothesis. This objection can only be met if we can show that the process of interchange, extending over a prolonged period, might take place by steps each of which would give the plant a biological advantage: the chance of a second accident following the first being thus as great as the original chance of accident.

This follows from the work of Cleland, Oehlkers and Håkansson in correlating their cytological observations with the genetical results of de Vries, Renner and others. Ring-formation in *Oenothera* has been shown to be associated with three genetic peculiarities: first, the formation of a single linkage group within the ring; secondly, the occurrence of a homozygote-eliminating mechanism similar in effect to the balanced lethal system in *Drosophila*; thirdly, an extremely heterozygous condition of the plant.

With ordinary pairing a balanced lethal system, such as is supposed to occur in one form or another in *Oenothera*, will preserve the heterozygosity of one linkage group, at most, one pair of chromosomes. By its functioning it reduces the potential fertility of the plant by one-half. To preserve the heterozygosity of a race in every separate chromosome it would be necessary to have seven balanced lethal systems in *Oenothera*. Each of these reducing the potential fertility of the plant by one-half, the whole seven would bring it to vanishing point, would in fact exterminate the race as sexually reproduced.

A balanced lethal mechanism with no crossing over in a self-pollinating plant will have the same effect as cross-pollination, facultative or imposed by self-sterility, namely, that in the species identical or related types cannot be brought together; these types are gametic in the balanced lethal species, while they are zygotic where self-sterility operates; in either case the result is the same, a permanently mixed zygotic condition[1].

Let us now consider the process by which de Vries' *Oe. muricata*, found by Cleland to have a single ring of 14 chromosomes, could have come into being. We have, to begin with, a form with seven pairs of chromosomes and with very few of the peculiarities we associate with

[1] Thus it will be observed the balanced lethal ring-forming combination is likely to have much the same relation to heterozygosity as dioecism, self-sterility, polyploidy, apogamy and other means by which a hybrid condition is encouraged or maintained.

the present heterozygous ring forms beyond a certain degree of hetero-
zygosity. It is possible that amongst the factors in which heterozygosity
occurred were some having a lethal or lowering effect on the sporophyte
in the homozygous condition. We may legitimately assume that the
maintenance of a heterozygous condition was of some value to this
original form, for there is no group in which the widespread occurrence
of heterozygosity and the means of preserving it have been more amply
demonstrated than in *Oenothera*. Two possibilities now offer themselves;
either we have segmental interchange giving rise to a ring of four chromo-
somes before the balanced lethal mechanism comes into existence, or
we have the balanced lethal mechanism developed before the ring. In
the first case the ring will only survive by means of a differential
activity in the pollen or embryo-sacs or by crossing; for self-fertilisation
under ordinary conditions will mean a chance halving of the new type
at each generation. This first possibility is however not at all improbable
for cross-pollination is very general amongst the natural species with
ordinary pairing[1]. In the second case the extension of the working of
the balanced lethal from two chromosomes to four means an increase
in its efficiency. In so far as it is an advantage to the plant in preserving
the hybridity of one pair of chromosomes its extension to two pairs will
therefore be a further advantage. The new type combining a balanced
lethal with a ring mechanism will thus be preserved, and when once
established hybridisation can only render it more and more a mechanism
for preserving the heterozygosity of the race in respect of the four chromo-
somes concerned.

    There is yet another possibility that we must not neglect. Where a
balanced lethal mechanism has arisen in *Drosophila* it has been found
to be associated with an inversion of a piece of chromosome. The
inversion *C III R* found in a balanced lethal stock had a definite geo-
graphical range in the wild species (Morgan, Bridges and Sturtevant,
1927). Further, translocation of "pale" in *Drosophila* was accompanied
by a deficiency, lethal in the homozygous condition. It is therefore not
inconceivable that a balanced lethal mechanism should have arisen in
Oenothera simultaneously with the accident of interchange. It must
be noted by the way that the occurrence of inversion in *Drosophila* in
association with balanced lethals would itself lead us to anticipate the
possibility of the structural changes we are supposing to have taken
place in *Oenothera*. There are thus several ways in which the formation
of the ring mechanism may have begun. It would be useless for the

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[1] In *Oe. grandiflora* with 7 bivalents according to Gates "open pollination" is the rule.

present to consider more closely which of these is the most probable, for the possible genetic conditions under which a new type could have arisen in this genus are themselves too numerous to be specified. It is enough if the accident of interchange is admitted to be possible, advantageous in its effect, and likely to be associated with the origin of a balanced lethal mechanism.

Beyond this stage any repetition of the original accident of interchange will extend the ring—two chromosomes at a time—and in so doing will extend the functioning of the balanced lethal mechanism. That the ring preserves the heterozygosity of the chromosomes taking part in it has been shown in some detail by Cleland and Oehlkers[1]. It follows that the original advantage is extended until we have the ring of 14 chromosomes.

By the simple operation of a balanced lethal mechanism the potential fertility would be reduced to one-half. Actually in *Oe. muricata* the lethal-plus-ring mechanism does not reduce it even to a half, for with nearly all the "active" pollen of the *curvans* type and nearly all the functional embryo-sacs of the *rigens* type, as Renner has shown, only a very small proportion of the embryos will fail to be of the viable hybrid combination. The development of a form with complexes having such opposite effects might thus contribute decisively to the success of the new mechanism.

So far we have seen that interchange with parasynapsis of homologous segments of chromosomes provides a plausible explanation of the occurrence of rings in *Oenothera*. We must now see whether the mechanism will function in accordance with observation, both genetical and cytological, in the "species," as well as in their mutants and hybrids.

The hypothesis rests on the principle that metaphase pairing results from the conjugation of homologous elements. The first special requirement that it has to meet is that the body of chromosomes going to each pole shall make up a full haploid complement. The arrangement of the rings at metaphase in *Oenothera* is such that the elements that we are assuming by their association to be homologous will always pass to opposite poles, and since no two whole chromosomes are alike we shall

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[1] Cleland, p. 557, states that "permanent heterozygotes have large circles," and later, p. 558, "the most striking peculiarity of the permanently heterozygous species of *Oenothera* is the fact that in each species the genes are, for the most part at least, associated into a single linkage group." On our hypothesis Oehlkers' view that the rings are responsible for the heterozygosity is more reasonable than Cleland's suggestion that the heterozygosity is responsible for the rings although, of course, the responsibility is not direct.

always have the same order; the same two sets will therefore separate just as the same two "complexes" segregate genetically. The ring will make, as Cleland has it, a single linkage group.

We have been assuming that reduction takes place at the first division except where crossing-over has occurred, for if the second division were reductional the separate chromosomes would segregate at random and the first division ring could have no bearing on the linkage between different chromosomes of the same complement. It is clear that if alternate numbers always pass to the same pole linkage will be effective only if they are the unaltered members of one parental complement; if, that is to say, only identical sister chromatids are associated and the division is therefore reductional. How is this related to the hypothesis? If interchange is responsible for the origin of the rings no two chromosomes in a ring are wholly identical. It is thus impossible to have—again apart from crossing-over—the association at metaphase of any other than sister chromatids between the point in each chromosome at which its homologies change and the chiasmata at which it meets the chromosomes with which it is paired. Since in *Oenothera* these chiasmata are always terminal, it follows that at the point of attachment of each chromosome to the spindle only sister chromatids will be paired, and the first division will therefore be reductional. Thus the behaviour of *Oenothera* in regard to the method of reduction is compatible with parasynapsis and the homology of segments.

To return now to the direct evidence of experiments:

A ring-forming type $AB-BC-CD...$[1] when doubled somatically should have its ordinary pairing $AB-AB, BC-BC...$ etc., partly restored and partly interfered with by the formation of trivalents ($BA-AB-BC$), quadrivalents ($BA-AB-BC-CB$), and other compound bodies, but the formation of rings found in the diploid while possible would certainly be unusual. This condition is probably fulfilled in *Oe. Lamarckiana gigas* (Gates, 1909; Davis, 1911); but owing to this tetraploid having arisen on various occasions through irregular germ-cell formation, what we might call its segmental constitution cannot be exactly forecast. No complete conclusions have been drawn with regard to the association of chromosomes in this tetraploid but it appears from Gates' description (1909) that pairing takes the place of ring formation: "The forces which lead to the regular

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[1] These symbols are the easiest to follow but they give a clumsier appearance than is necessary, for they seem to imply that every chromosome consists of two parts that have been separate; actually with a ring of fourteen only the seven of one complement need have been affected by interchange.

alignment of the chromosomes in two parallel series are evidently stronger than is usually the case in *Oenothera*, for in the *Oenothera* forms studied usually no such regular alignment takes place." Håkansson (1927) describes and illustrates pairs and quadrivalents as well as short rings of the diploid type; the last though incomplete are probably very uncommon. At the same time the balanced lethal mechanism would cease to operate and would also to a great extent lose its virtue, for the heterozygous condition would be maintained by the pairing and segregation of likes.

The trisomic forms afford another cytological test. It will be noticed that, on the interchange hypothesis, the formation of a ring with an odd number of chromosomes is possible, *e.g.* $AB–BC–CA$. Yet while this has been observed by Cleland for example in the trisomic mutant *oblonga*[1] it has not been found in diploids. This follows from the hypothesis in this way: rings must be genetically disomic although composed of an odd number of chromosomes. Thus $AB–BC–CA$ is a ring of an odd number of chromosomes but it is genetically disomic in each of the segments A, B and C. Hence in a diploid a single odd-numbered ring is impossible, for the ring would be disomic while the chromosomes outside the ring would be present in odd number and the whole complement would therefore be trisomic in one chromosome and monosomic in another.

In the form *oblonga* Cleland found sometimes a ring of three, sometimes a chain of 5 or 7. Such a trisomic type would be expected to arise as a result of interchange within the ring; thus if the ring in *Oe. Lamarckiana* is $AB–BC–CD–DE–EF–FG–GH–HK–KL–LM–MN–NA$ and the reduplicated chromosome is CA (resulting from interchange) then we shall have either a circle of three, $CA–AB–BC$, as above or a longer chain, not a circle, thus: $CA–AB–BC–CD–DE$, and so on. From the fact that a complete chain was never observed it would appear that the chain is less stable than a ring; nor is this surprising, for while any two parts of a chain are only joined by a single connection, a double connection joins any two parts of a ring.

It is necessary now to consider certain very definite results from hybridisation. For example *biennis* with a circle of 8 and a circle of 6 crossed by *Hookeri* with no circle gave three plants with a circle of 10 and 2 pairs and five plants with a circle of 14. This condition is met if we take *Oe. biennis* with two rings as $AB–BC–CD–EF–FA$ and

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[1] Such a combination in an immediate derivative of *Lamarckiana* indicates that further segmental interchange has been associated with its origin. The association of three to be expected in a trisomic mutant of *Lamarckiana* AB, BC, BC, with the three B's associated at one point, might however conceivably be described as a ring.

GH–HK–KL–LM–MN–NO–OP–PG, while *Oe. Hookeri* without rings would be *OB–OB, CD–CD, EF–EF, GH–GH, KL–KL, MN–MN, AP–AP*. The progeny should be of four types, one with a ring of 4 and 5 pairs, one with a ring of 8 and 3 pairs, one with a ring of 10 (*GH–HK–KL–LM–MN–NO–OB–BA–AP–PG*) and two pairs (*CD–CD* and *EF–EF*) and one with a ring of 14. It is interesting to note that in a small family only the last two types occurred, being those with the greatest size of ring, for according to Oehlkers' and Cleland's conclusions and our hypothesis, rings should be most heterozygous; according to our view, in a self-fertilised plant on account of the separation of homologous elements by the balanced lethal, and in a hybrid owing to the fact that only related chromosomes will form pairs, so that plants with pairs will be more likely to be of the types eliminated by homozygous lethals. This is more or less in accordance with Oehlkers' view. Thus, when the *biennis* of the formula given above is crossed with *Lamarckiana* (*AB–BC–CD–DE–EF–FG–GH–HK–KL–LM–MN–NA. OP–OP*) theoretically eight forms could arise; of these one would be the ring-of-twelve form like the *Lamarckiana* parent; the others would have smaller rings and more pairs, and being more homozygous would be more likely to be eliminated. The ring-of-twelve form was found · by both Håkansson and Cleland; the others were not.

To take another case. Oehlkers (1926) crossed *Oe. suaveolens* by *Oe. strigosa* and obtained one type with seven pairs (*flava*) and one type with a ring of 12 and one pair (*albata*); the former split up into a number of forms, the latter bred true. This is intelligible on our view if we represent one of the parents as ring-forming *AB–BC–CD...* with balanced lethals, and the other as either similar or pairing normally *AB–AB, CD–CD....* From these we should expect two sorts of offspring; the one ring-forming and, if with balanced lethals, breeding true, viz. *albata*; the other with simple pairing would appear only if the same lethals were not present in the similar sets *AB–AB, CD–CD* of the two parents, and we should then have a simple pairing form like *flava*. As it is unlikely that every chromosome would carry lethals, segregation of interspecific differences would show in the next generation.

A cross between two ring forms *AB–BC–CD...* in which the same lethals were not involved would give viable *AB–AB, CD–CD...* or *BC–BC, DE–DE...* forms with simple pairing only, although when either ring form was selfed the balanced lethal would operate to prevent such combinations occurring; thus the ring forms would be capable of giving individuals with simple pairing, lethals permitting and a

particular cross would give specific results in regard to the linkage properties of the offspring. Renner has shown this specificity by the most elaborate experiments many of which provide a test of our hypothesis. For example, the factor R segregates independently of *velans* and *gaudens* in *Lamarckiana*; it must therefore be located in the free pair, *OP–OP.* When *Lamarckiana* is crossed by *muricata*, R is completely linked in the new *curvans-velans* combination. This is accounted for if we take *velans* to be *AB, CD, EF, GH, KL, MN, OP, gaudens BC, DE, FG, HK, LM, NA, OP*, and *curvans BC, DE, FG, HK, LM, NO, PA*; then *velans* and *curvans* will form a complete ring and the odd *OP* chromosome will, for the time being, form part of the *velans* complex. Similarly, without giving the explanation by formula in each case, we can show that a definite and constant segmental constitution will account for the occurrence of complete linkage in *rigens* when opposite *curvans* and its disappearance when opposite *Hookeri*; and for the fact that the factors B and M, linked in *curvans* when opposite *rigens*, can be crossed over to *rigens* by first crossing them over to *flavens*. These are mere instances of the operation of a system which for precision and constancy can only be compared with *Drosophila*. The examples that have been worked out show that the hypothesis of interchange is capable of providing a physical basis for this system, is capable of co-ordinating the genetical and cytological behaviour of *Oenothera*. They also serve to strengthen the hypothesis in showing that we can forecast cytological and hence genetical results in a way that would otherwise be scarcely possible. But it does not seem necessary to go more deeply into its implications for the moment. Experiments undertaken with the idea of parasynapsis and interchange in view will doubtless provide a more searching test than any of which the results are at present available.

NOTE ON *TRADESCANTIA*.

The possibility of finding evidence on the relation of the formation of rings to the homology of their constituent chromosomes led me to investigate *Tradescantia virginiana* and some of its relatives, in which ring formation following parasynapsis had been described by Miyake (1905) and others. This interpretation is confirmed; prophase behaviour is only compatible with parasynapsis; exceptionally, perhaps only in certain varieties, chromosomes are associated otherwise than terminally, but as a rule, and always within the rings, association is by terminal chiasmata.

Here evidently are chromosome arrangements both of the diploid type and of that characteristic of the tetraploids (compare diagram). Thus in some cases the longest strings may divide so that, as in *Oenothera*, we have the regular separation of alternate chromosomes to opposite poles. In other cases, however, owing to four chromosomes being terminally associated at one point, this is impossible. Also a ring of four is frequently formed in which adjoining chromosomes pass in pairs to the same pole.

In order to find out if this was due to a truly tetraploid condition a number of related species were studied for the somatic chromosome number. Root-tip counts gave the following results:

Tradescantia virginiana	$2n = 24$
T. navicola	$2n = 32$
T. fluminensis	$2n = 60$[1]
Tinantia fugax	$2n = 68$
Rhoeo discolor	$2n = 12$[2]
Commelina coelestis	$2n = 90$
Cyanotis somaliensis	$2n = 28$

The chromosomes of *Rhoeo discolor* and *Tradescantia crassifolia* ($n = 6$) are comparable in size and form to those of *T. virginiana* and it is therefore reasonable to assume that this species is tetraploid, and that in its diamond-shaped rings the pairs that fail to separate are homologous chromosomes.

But this will not explain the association of chromosomes in other forms and in numbers greater than four. Moreover, there are other grounds for believing that the chromosome constitution of *Tradescantia* has been affected by an exceptional process of another kind.

First, single chromosomes occur at somatic divisions, for which no identical mate can be found: secondly, as a result of this, unequal pairs occur at the reduction division: thirdly, in the first division of the microspore, fragments which have appeared at meiosis develop attachment constrictions which enable them to pass to opposite poles in mitosis and give them the phylogenetic importance of ordinary chromosomes. This fragmentation naturally suggests itself as the cause of the unequal pairs, for side by side with the fragments can be seen the chromosomes which have been reduced by their loss. Its occurrence is probably to be associated with the exceptional methods of chromosome pairing which

[1] Tischler (*Allgemeine Pflanzenkaryologie*) reports $n = 12$.
[2] Reported by Suessenguth (1920, 1921), and Tischler (1921).

must precede the formation of strings at metaphase, as appears to be the case in *Hyacinthus* and *Tulipa* (Newton and Darlington, in the press). But the occurrence of fragmentation is interesting because it has also been found in *Oenothera* (Gates and Thomas, 1914; Lutz, 1916; Hance, 1918). In *Tradescantia* also evidence is not wanting that this fragmentation is of evolutionary significance, for in the related forms with more than 24 chromosomes these frequently have the diminutive size and characteristic shape of fragments; their mass as a whole is probably no more than equal to that of the 12 chromosomes of the diploid species. It is therefore possible to consider fragmentation as connected, either as cause or consequence, with abnormalities in segmental homology.

In *Rhoeo* a closed ring of twelve chromosomes is formed and behaves in every way like a comparable ring in *Oenothera*. The remarkable behaviour of *T. virginiana* is explained on the hypothesis of parasynapsis and interchange if we regard it as the result of chromosome doubling in a form like *Rhoeo*; in other words, as comparable with *Oenothera Lamarckiana gigas* (see p. 7). On this hypothesis—but scarcely on any other—we should be led to anticipate utter inconstancy of arrangement owing to each end of each chromosome having three ends homologous with it and the two ends of each having homologies with different chromosomes. Thus we should expect to find the association of three or four chromosomes terminally at one point as well as the formation of longer chains of chromosomes; we should expect closed rings of even numbers of chromosomes but not of odd numbers; we should expect occasional unpaired chromosomes. Innumerable types of combination have been found, all corresponding to these expectations. The segmental constitution of *T. virginiana* (or at least of some forms of it) can therefore be regarded as not departing very far from the simple formula:

AB BC CD DE EF FG GH HK KL LM MN NA
AB BC CD DE EF FG GH HK KL LM MN NA

In *Tradescantia* and *Rhoeo* the view that parasynapsis and interchange are responsible for ring-formation provides a useful working hypothesis.

RECAPITULATION.

There are many reasons for believing that the side-by-side pairing (parasynapsis) of homologous chromosomes, or parts of chromosomes, at prophase is the essential condition of meiosis in plants. These will be given in detail in later papers on *Tulipa, Hyacinthus, Fritillaria* and *Tradescantia*.

Of direct evidence for the alternative theory there is none. Telosynapsis is *inferred* from the indisputable facts of observation at diakinesis. The hypothesis of parasynapsis is *inferred* from the same facts. The difference between the two views is therefore a difference of reasoning and not of observation.

The principle of parasynapsis is applied strictly to the ring-forming *Oenothera* species and it is found necessary to assume that segmental interchange of the kind described in *Datura* has taken place between seven complementary chromosomes of the fourteen present. This view is strengthened by the observation in both *Oenothera* and *Tradescantia* of fragmentation which, like interchange, is an accident to the structure of the chromosome, and by the behaviour of *Tradescantia* which is such as on this hypothesis would be expected of a tetraploid relative of *Rhoeo discolor*.

A ring of fourteen chromosomes formed after interchange will consist of two groups either of which meeting with one like itself will form seven pairs, freely segregating. The ring itself combined with a balanced lethal mechanism establishes, primarily, two independent linkage groups, and secondarily, a genetic differentiation between them.

It is suggested, first, that this result could be attained by a series of steps, each of which would be of advantage to the race; secondly, that the behaviour of individual plants, as shown by both genetical and cytological study, agrees with the assumption of interchange necessary to explain their particular condition; thirdly, that the progeny from crossing and selfing the heterozygous species give results agreeing with this assumption.

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APPENDIX

Since going to press I have received an account by Håkansson of meiosis in the embryo-sac in *Oenothera* (*Hereditas*, XI). Håkansson points out some of the advantages of applying Belling's conclusions with regard to segmental interchange in *Datura* to the solution of the *Oenothera* problem. At the same time he apparently finds in his own observations

of the behaviour of the "Halbmutanten," as well as in the telosynaptic theory, a difficulty in the way of accepting interchange as a working hypothesis. The mutants of *Lamarckiana* that are in question have a ring of six chromosomes and four pairs; they arise, according to Håkansson, from irregularities in the arrangement of the ring on the spindle, as a result of which adjoining chromosomes pass to the same pole. With an even number of chromosomes in the ring this non-disjunction must necessarily happen twice and where it occurs on opposite sides and, on the old theory, between non-homologous chromosomes, it is possible to get two new haploid sets from such an arrangement. For example, if *abcdef* is the *velans* set, *ABCDEF* the *gaudens* set, and *GG* the pair, then we shall have gametes such as *abcDEF,G* and *ABCdef,G* which, with normal gametes, will give in the zygote a ring of six and four pairs. The occurrence of this non-disjunction thus offers a neat explanation of the Halbmutanten, *on the old theory*, with the one difficulty that the Halbmutanten are rare while non-disjunction is very frequent. It is common not only in *Lamarckiana*, but in all other forms with large rings. This is indeed to be expected; for irregularities are evidently the result of the establishment of relationship with the spindle of different parts of the ring at the same time, which must result in there being an equal chance of normality and non-disjunction in distribution at anaphase.

Rhoeo affords better material for the study of these irregularities. Belling (*Jour. Gen.* XVIII) has shown here a ring of chromosomes at metaphase and the interchange hypothesis must therefore be taken to apply. The present observations have shown that this ring is subject to exactly the same irregularities of distribution as those described by Cleland (1926) and Håkansson (1928) in *Oenothera*. Here they are more frequent, perhaps on account of some of the chromosomes having more or less subterminal attachments. The irregularities result in the formation of pollen grains with five and seven, as well as with the normal six, chromosomes. (At the mitotic division of the pollen grain only the normal type and that with the extra chromosome are represented; that with five chromosomes, apparently, does not survive so far.) This numerical irregularity results from both non-disjunctions being on one side, as I have observed them in *Rhoeo*, and as Kihara has found (1927) in *Oenothera*. On any hypothesis the $n + 1$ gametes so formed should be viable, the $n - 1$ non-viable.

Now, as Håkansson justly points out, the Halbmutanten, having *arisen* in the way he has suggested, on the basis of the old theory, should

not *behave*, if we accept the new theory, as they actually do behave: they should form an open chain and not a ring at diakinesis. This he takes to be an objection to the new theory; but surely it is rather an objection to this method of reasoning. For, while, on the old theory, as Cleland has shown in detail (1926)[1], non-disjunction on opposite sides of the ring could give rise to both viable and non-viable types of gametes, on the interchange hypothesis, on the other hand, viable gametes cannot so arise; each non-disjunction means a deficiency on the opposite side which is deprived of the non-disjoining segments. Håkansson admits this in saying that the mutants would be haploid in one segment and triploid in another; in other words, to produce such a type a genetically defective gamete would need to be viable, *quod est absurdum*.

Thus Håkansson's theory of the origin of the Halbmutanten is incompatible with the interchange hypothesis.

But need we regard non-disjunction as providing the only explanation, plausible though it may seem to be, of the origin of the Halbmutanten? Taking *Lamarckiana*, as we did before, to be $AB_1—BC_1—CD—DE_1—EF—FG_1—GH—HK_1—KL—LM_1—MN—NA, OP—OP$, then a further occurrence of interchange between non-corresponding segments of opposite complexes, as, for example, between A_1 and C_1, G_1, K_1, or M_1, will cause the two complexes to segregate abnormally, part of each going to one pole and part to the other. Thus, interchange between A_1 and G_1 will result in the segregation of the two new types of gametes: $G_1B, CD, EF, AN, ML, KH, OP$ and $BC, DE, FA_1, NM, LK, HG, OP$. These gametes are of mixed constitution in regard to *velans* and *gaudens* and either kind combined with either *velans* or *gaudens* will give a new type with a ring of six and four pairs—a Halbmutant.

In this way the hypothesis of interchange explains the *origin* and *behaviour* of the Halbmutanten. But it does more; it requires their *occurrence* as a normal, if uncommon, feature of the behaviour of ring forms; for, if these have arisen by interchange, there is no reason to think that this mechanical accident will cease to occur when a particular biological effect has been attained.

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[1] Cleland's text-figures 4–6; fig. 7 shows the same theoretical possibility as fig. 6.

THE GENETICS OF COTTON. PART I. THE IN-HERITANCE OF PETAL SPOT IN NEW WORLD COTTONS.

By SYDNEY CROSS HARLAND.

(With One Colour Plate and One Text-figure.)

CONTENTS.

INTRODUCTION.

IN common with some other genera of the Tribe Hibiscideae, in the Natural Order Malvaceae, the flowers of *Gossypium* usually possess a spot or eye at the base of the petal. In both Old and New World cottons, varieties are known without spot, but in Old World cottons the absence of spot is a manifestation of the general absence of anthocyanin pigmentation from the plant body, for when spot is present the stem and other parts of the plant body are reddened, and when absent the whole plant is green. Even when spot is absent there is often an area, sharply delimited, of the same shape and size as the normal spot, unpigmented but occupying exactly the same position, which may be termed a "ghost" spot. The spotless Asiatic is thus analogous to the "albus" form of *Hibiscus Sabdariffa* described by Howard (1924). The New World cottons show a series of grades of spot, grading from a most intense purple blotch occupying almost all the lower quarter of the petal, to a spot which can scarcely be seen with the naked eye, consisting indeed of only two or three pigmented cells at the base of the petal. The Asiatics also show variations in the intensity of the spot, but the tremendous variety of intermediates characteristic of New World cottons is lacking.

In the cultivated varieties of *Hibiscus rosa-sinensis* various kinds of spot may be noted, but there is no continuous gradation down to spotless.

Investigations on the inheritance of petal spot have been carried out by Balls, Kearney, McLendon, and Harland in New World cottons, and by Leake and Ram Prasad in Asiatic cottons.

Balls (1910, 1912) worked with Egyptian-Upland hybrids. Egyptian full spot by Upland spotless gave an intermediate F_1 and ratios of 23 : 42 : 31, and 11 : 22 : 18, in respect of Full : Intermediate : Spotless occurred in F_2. The ratios in four small F_3 families were erratic. In a later publication he states that spotless breeds true, while full may either breed true or break. The intermediates are said to break in at least two different ways. He considers that probably two allelomorphic pairs are involved.

McLendon (1912) reported that in crosses of full spot Sea Island with Upland spotless, the F_1 produced an intermediate spot, with F_2 progenies varying greatly in the proportion of spotted to spotless. In most of the families the proportion of spotless individuals was in excess of that demanded by a 3 : 1 ratio.

Harland (1915) states that in a cross between St Croix Native (spotless) and Sea Island (full spot) the F_1 was intermediate, while the F_2 showed segregation into a range of types grading from full spot down to spotless. It was noticed that "many of the types which appeared at first to be spotless have on careful examination shown an extremely faint spot. Four petals out of the five may be spotless and the fifth show a faint trace of colour." In F_2 the ratio 67 spotted to 15 spotless was obtained.

Kearney (1923) studied an Upland-Egyptian cross. Segregation took place in F_2 into 152 spotted and 63 spotless, expectation being 161 to 54 on a 3 : 1 basis. 22 F_3 families were grown, but the results were erratic. Combining all the segregating families gave 180 spotted to 88 spotless. The author states: "This is far from being a 1 : 3 ratio, the deviation from the expected percentage of spotless having been $7 \cdot 8 \pm 1 \cdot 9$ per cent.; but in view of the likelihood that the number of spotless individuals would have been found to be smaller if larger numbers of flowers had been examined, the supposition that complete absence of the spot, as compared with its presence, is a simple recessive character cannot be regarded as disproved."

By way of comment it may be said that the numbers obtained in segregating F_3 families are far too divergent, there being ratios of spotted to spotless such as 3 : 7, 16 : 16, 18 : 19, 22 : 1, etc.

The same author (1924) found in a field of commercial Pima cotton (Egyptian) two plants with a very faint spot. He crossed these plants, which bred true to the reduced spot, with normal full spot Pima. He obtained clear segregation into strong and weak spot:

	Full	Weak
	130	49
Expected	134·25	44·75

The F_3 results were consistent with those obtained in F_2, and in segregating families 249 spotted to 90 weak spotted were obtained. It is thus clear that the weak spotted variety of Pima is allelomorphic to the full spot type, and it may be suggested that it is in the nature of a simple recessive mutation.

This case of Kearney's, so far as the present writer is aware, is the only one in which petal spot has been observed to segregate in simple Mendelian fashion, and it forms the only case in which the mode of inheritance of petal spot has been followed in a varietal form of a species. All other cases have been in inter-specific crosses, where complicated results might naturally be expected.

Leake and Ram Prasad (1914), working with Asiatic cottons, state that all true Indian cottons possess a spot at the base of the petal. In some forms from China the spot was absent, and crosses between these types and an Indian gave a fully developed eye or spot in F_1 with 29 full spot : 10 no spot in F_2. They suggest that possibly a single factor difference is involved, though the evidence is incomplete. An intermediate form of eye was found to breed true.

Reviewing the information on petal spot and its mode of inheritance, it is clear that the range of types is greater in New than in Old World cottons, and probably the mode of inheritance more complicated. The connection of a simpler type of inheritance with the lower chromosome number, 13 pairs of chromosomes as against 26 pairs—may be emphasised but not unduly stretched. So far it appears that inheritance in inter-Egyptian crosses (Kearney) may be straightforward, but that complicated results, not explicable on a simple unifactorial basis, occur in inter-specific hybrids.

THE PRESENT INVESTIGATIONS.

From previous experience with the hybrid St Croix Native × Sea Island in which many gradations of spot were found, it was felt to be necessary to establish a number of grades covering the whole expected range of types. At first 22 grades were made. These were painted and

used as standards (see Plate IX). Three flowers were examined from each plant, and as in Kearney's experiments, the aim was to indicate the amount of anthocyanin pigment present, rather than size or intensity alone. The grades established thus integrate size and intensity, which are correlated, though not altogether completely. After making 22 grades, it was discovered that some flowers were below grade 1 and not entirely spotless. Two more grades were therefore made, which were called ·25 and ·50. The grade ·25 is exceptionally minute, and usually consists of a cluster of two or three cells with anthocyanin pigmentation. The spot was measured on one occasion and found to be ·07 mm. long by ·04 mm. broad. It is clear that there is no definite line of demarcation between spot and spotless, and the lowest grade of spot must consist of a single pigmented cell. The most extreme case yet encountered was a plant which gave a small proportion of spotted progeny, up to grade 1, but in 25 flowers produced only one flower with the slightest trace of spot (·25). The interesting point, however, is that most commercia varieties of Upland never produce even this minute trace of spot, being spotless under all environmental conditions under which they have been observed. One type of Upland—Dominant Naked—produces spots up to grade ·50.

To sum up: New World cottons comprise all grades of spot, from a large purple blotch which occupies the whole claw of the petal, to a single pigmented cell, the latter being frequently extinguished by fluctuation. Thus, at the outset of the experiments, we are in ignorance whether "spotless" really exists at all, or whether in all cases we are merely dealing with more spot or less spot.

TABLE I.

Spot grades in homozygous families

	0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Mean
Upland Meade	*																								0
Dominant Naked	15	5																							0·06
Cauto	1	1	3	3	1																				0·75
TRK[1]				1		1	22	9	6	3															4·6
Pima Spotless											1	3	2	22	17	12									11·5
SIW												3	5	10	32	4									11·5
GWP													1	2	4	3									11·9
V 135															1	1	2	2	3						14·6
SIN																		2	6	2	1				16·0
GN																		1	5	5	1				16·5
Pima Full Spot																					2	5	16	4	19·8

[1] For the meaning of these abbreviations see below.

The types worked with may be roughly grouped as follows:

Spotless.	Upland, Cassava, Trinidad Red Leaf.
Very faint spot.	Upland Dominant Naked.
Faint spot.	Cauto, Jamaica Long Staple.
Weak spot.	Trinidad Red Kidney.
Intermediate spot.	Grenadines White Pollen.
Full spot.	Pima, Sea Island, Grenadines Naked, Bourbon Intense spot.

Frequency arrays of spot for certain standard types are to be found in Table I.

MATERIAL.

Brief notes on the material used in the experiments are placed below:

Type	Group	Symbol	Remarks
Upland	Upland	*U*	Ordinary American Upland, *G. hirsutum* L.
Cassava	Bourbon	*CAS*	Trinidad Native. Laciniated leaves, *G. purpurascens* Poir
Trinidad Red Leaf	,,	*TRL*	Trinidad Native. Red leaves, *G. purpurascens* Poir
Cauto	Peruvian	*CAU*	Native of Cuba. *G. brasiliense* var. *apospermum* Sprague
Jamaica Long Staple	,,	*JLS*	Similar to Cauto, differing only in minor characters
Trinidad Red Kidney	,,	*TRK*	Trinidad Native Red Kidney cotton. *G. brasiliense* Macf.
Grenadines White Pollen	,,	*GWP*	A type of commercial Marie Galante from the Grenadines
Pima Weak Spot	,,	*PWS*	Pure line of weak spotted Pima Egyptian, isolated by Kearney in Arizona
Pima Full Spot	,,	*PFS*	Pure line of full spotted Pima Egyptian, isolated by Kearney in Arizona
Sea Island Yellow	,,	*SIY*	Pure line of Sea Island isolated by author in St Vincent
Sea Island White	,,	*SIW*	A white flowered mutant from Yellow. A pure line

THE EXPERIMENTAL RESULTS.

1. Upland (spotless) × Trinidad Red Kidney (weak).

The results of this cross are presented in Table II.

From the above results it will be seen that segregation takes place in F_2 into spotted and spotless, and that the grade of spot exceeds *TRK*, in some plants. It is not possible, however, to divide the frequency array at a point of minimum frequency, so as to indicate an allelomorphic pair of characters. Early in the investigation, however,

TABLE II.

Results of the cross Upland × Trinidad Red Kidney.

	Family		0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12	13
	Upland		*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	TRK		.	.	.	1	.	1	22	9	6	3	.	.	.	.	.	.
	F₁		.	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.
F_2	555	R	36	12	3	6	6	6	1	.	2	1	3	.	2	.	1	.
		r	27	2	1	2	2	.	.	1	1	.	.	.	.	.	.	.
	556	R	42	8	6	3	5	6	1	2	1	.	1	.	4	.	1	.
		r	31	6	1	.	.	1	.	.	.	.	1	.	.	.	.	.
	557	R	3	5	1	1	1	2	.	.	.	.	.	.	.	.	.	.
		r	2	1	1	.	•.	.	.	.	.	.	.	.	.	.	.	.
	558	R	94	23	5	12	4	.	3	.	2	1	1	.	.	.	.	1
		r	37	4	5	.	.	.	.	.	.	.	.	.	.	.	.	•
	559	R	102	26	8	13	10	7	3	.	2	3	4	.	1	1	.	.
		r	58	13	4	.	.	.	.	.	.	.	.	.	.	.	.	.
	560	R	26	4	1	5	.	1	.	1	.	.	.	.	.	.	.	.
		r	6	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	Total	R	303	78	24	40	26	22	8	3	7	5	9	.	7	1	2	1
		r	161	31	13	2	2	1	.	1	1	.	1	.	.	.	.	.

it was discovered that the gene R^1, which produces strong anthocyanin pigmentation of stem and leaf, is linked with spot in all crosses. For this reason the frequency arrays for **R** and **r** have been plotted separately in the above families. It is safe to say that *TRK* brings into the cross a spot gene which is linked with **R**, and that spot of *TRK* is allelomorphic to a less spotted condition. It will be noticed that few **r** plants are found with a spot grade above ·50, so that *TRK* has introduced one or more modifiers which enable the recessive of the main gene to rise to this level. It may be hazarded further that the main gene in the **R** class extends downwards into the 0 class—extinguished by fluctuation.

The fact that the grade of spot in F_2 rises above that of *TRK* indicates also that Upland introduces a modifier, or modifiers, capable of intensifying the *TRK* spot.

As the frequency arrays stand it is not possible to delimit exactly the four classes **RS, Rs, rS, rs**. It seems as if class ·50 may be the dividing line in the **r** class, since there is a sharp change of ratio from 24 : 13 in grade ·50, to 40 : 2 in grade 1. The ratio **rS : rs** is thus 8 : 205 indicating 1·9 per cent. cross-overs. Similarly accepting all **R** plants above grade ·50 as positively **RS**, we get the ratio **RS : rS** of 131 : 8, indicating 9 per cent. cross-overs. The first value is probably too low and the second too high, and the real value may be somewhere

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[1] The genetics of anthocyanin coloration will be dealt with in a subsequent paper.

in between—5·5 being the arithmetic mean, this value may provisionally be accepted.

2. Upland (spotless) × Cassava (spotless).

This cross is interesting in that it provides the unexpected result of spotted plants in the second generation, when both parents are spotless. The results, analysed separately for **R** and **r**, are to be found in Table III.

TABLE III.

Results of the cross Upland × Cassava.

	Family		0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12
	U		*														
	CAS		*														
	F_1		11	3	1												
F_2	414	R	143	16	1	1	2	2									
		r	55	10													
	416	R	72	12	3	6		1	2	1			2	1		1	
		r	30	8			2	1									
	417	R	49	10	5	5		2						1			
		r	23	2	2	1	1										
	418	R	60	17	8	3		3	2					1			
		r	25	4	4		1										
	419	R	57	13	5	2	2	1	1								
		r	20	5	1												
	420	R	78	10	2												
		r	23	2			1										
	Total	R	459	78	24	17	4	9	5	1			2	3		1	
		r	176	31	7	1	5	1									

This is a more extreme case of the phenomenon seen in the previous cross, where in the spotted parent the spot was frequently extinguished by fluctuation, and where the action of a definite spot gene was traced by means of the linkage which it exhibited with the gene **R** (Red). Although Cassava has no spot, the F_2 frequency arrays show clearly that not only do spotted plants occur, but that spot and red are linked, as in the previous cross. The conclusion is that since Cassava introduces red and since the linkage is of the coupling type, the spot gene is introduced by Cassava. In the presence of a favourable grouping of modifying factors the spot of Cassava comes to light, showing the characteristic linkage with **R**. The invisible spot of Cassava may be designated Extinguished Spot. It is, of course quite hopeless to expect a simple 3 : 1 ratio for the allelomorphic pair, spot and less spot (or extinguished spot and no-spot), since there is segregation presumably also for modifying factors, and until a type is obtained homozygous for all genes promoting the expression of spot, the 3 : 1 ratio will not appear. Even then it may not do so.

Family 420 appears to be somewhat different in genetic composition from the rest, in that the spot grade does not rise above grade ·50 in the **R** class. It seems as if the spot gene linked with **R** had not been brought into expression in this particular plant—presumably because one of the parents was heterozygous for one or more modifiers. The recessive of extinguished spot may be intensified up to at least grade ·50 by modifiers.

3. Cassava (spotless) × Pima (full spot).

This is a cross of extinguished spot by full spot. The results are presented in Table IV. The main conclusions to be drawn are as follows:

TABLE IV.

Results of the cross Cassava × Pima.

		Grade of Spot																						
		0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Pima																						2	5	16	4
Cassava		*																							
F₁															1		1								
677 E	R	1													1										
	r																						1		
678 E	R	1																1							
	r																						1		
679 E	R	2					1	1							1	1	1	3							
	r															2	1		2	2				1	
680 E	R	1																							
	r																								
682 E	R																1								
	r																								
684 E	R		2			1	2						1		1	1		2			3	1	1		
	r														1		1	1			1				
Total	R	5	2			1	3	1					1		3	2	2	6			3	1	1		
	r														1	2	2	1	2	2	1		2	1	

(*a*) A simple segregation into full spot and weak spot is shown, the ratio being 32 : 12.

(*b*) Full spot is thus allelomorphic to weak spot, the latter really being extinguished spot raised to as high as grade 4 by modifying factors brought in by Pima.

(*c*) It was previously seen that extinguished spot was linked with **R**, and that this linkage was of the coupling type. By reference to the table it is found that this linkage with **R** still exists, but is now changed to a repulsion:

Red full spot	Red weak spot	Green full spot	Green weak spot
17	12	15	0

(*d*) This switching over of the linkage type from coupling to repulsion can only be due to the fact that we are dealing with a system of multiple allelomorphs:

S^o Spotless.

S^e Extinguished spot.

S^f Full spot.

Each of these types can be modified to a greater or less extent by minor factors, and if, as is suspected, more members of the multiple allelomorph series exist, it will be rather difficult to identify them. If it is possible, by repeated back-crossing to the highest member of the series, to obtain the maximum expression of spot in all the members of the series, the change in the type of linkage will show whether the difference in intensity of spot in two given types is due to allelomorphism or to modifying factors.

4. Trinidad Red Kidney (weak) × Sea Island (full).

The results of this cross will be found in Table V. The following are the main points:

TABLE V.

Results of the cross Trinidad Red Kidney × Sea Island.

Grade of spot

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Sea Island	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	2	.	.	.	.
TRK	.	1	.	1	22	9	6	3	.	.	.	.	.	.	.	.	.	.	.	.	.
F_2 515 R	.	1	1	4	4	1	1	1	1	1	.	4	7	3	9	8	5	2	1	.	.
r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	5	2	1	3	2	.

(*a*) In the last cross it was seen that the linkage of **R** with extinguished spot was converted into a repulsion by crossing extinguished spot with full spot. In this cross an analogous phenomenon is seen, the weak spot of Red Kidney taking the place of extinguished spot of Cassava, being allelomorphic to full spot. The ratio full spot to weak spot is 53 : 15, with expectation 51 : 17 on a 3 : 1 basis.

(*b*) Reference to the table will show that the distribution of weak spot in the F_2 of Red Kidney × Sea Island is from grade 1 to grade 9, as against grade 0 to grade 4 in Cassava by Pima.

(*c*) It is not known whether the weak spot of Red Kidney is allelomorphic to the extinguished spot of Cassava, or whether it is the same gene plus modifiers. Crossing Cassava with a green plant of the same grade of spot as Red Kidney would give this information. If the F_2 ratio exhibited repulsion allelomorphism between weak spot and extinguished spot would be indicated.

5. 10 B 27 (heterozygous full spot) × Trinidad Red Leaf (spotless).

The plant 10 B 27 was (Upland × Egyptian) × Upland × Upland, and was spotted. When crossed with *TRL* spotted and spotless plants were produced. One of the spotted plants was back-crossed on a large scale to indicate, if possible, the strength of the linkage between **R** and **S**, and four spotted plants were selfed.

The results for these four plants are placed in Table VI below, and may be briefly commented upon.

TABLE VI.

Results of the cross 10 B 27 × *TRL*.

Grade of spot

Family		0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10 B 27												1							
TRL		*																	
541	R	32	4			2			1										
	r	5	2																
542	R	14	5			2	2	2		1	2	3	1	1					
	r					1					1	1	2	3					
543	R	5			1	1	1		2		1	3	2	1	1				
	r	1							1	1		4		1	1				
545	R	30			4	1	2		8	2	2	3	6	6	4	6	2	1	1
	r									3	4	1	4	6	1	8	2	3	2
Total	R	81	9		5	6	5	2	11	3	5	9	9	8	5	6	2	1	1
	r	6	2			1			1	4	5	6	6	10	2	8	2	3	2

(*a*) All the families show repulsion between red and a spot factor of the familiar kind, though segregation is not so sharp as in the Pima × Cassava, or Sea Island × Red Kidney crosses.

(*b*) The spread of the spotted group is markedly different in the different families. Families 542 and 545 are strongly contrasted. The former has a preponderance of spotless plants, and the range of spot does not extend above 5. In family 545 the spotted plants extend up to grade 15. Differences in modifying factors are no doubt responsible for differences in the type of segregation.

(*c*) The results of the back-cross with Upland, presented in Table VII, are more illuminating, for they enable the linkage between **R** and **S** to

TABLE VII.

Results of the back-cross Upland (**rs**) × (10 B 27 (**RsrS**) × *TRL*).

Grade of spot

	0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12	13
R	588	60	24	12	1	4	2	1	1	3	2	5		2		
r	24	9	1	12	9	20	24	24	43	53	68	111	98	79	27	2

be calculated. It is a little difficult to decide the limits of the four classes, but grade 1 is clearly the point at which the reversal of ratio takes place. Missing this class out the results are as follows:

RS	Rs	rS	rs
21	672	558	34

indicating a percentage of cross-overs of 4·3.

6. Pima (full) × Grenadines White Pollen (intermediate).

This is a cross of full spot by intermediate spot. The gene **R** does not enter into the cross. The results (Table VIII) show:

TABLE VIII.

Results of the cross Pima × GWP.

								Grade of spot								
Family	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Pima	.	.	.	.	.	.	.	.	.	.	.	.	2	5	16	4
GWP	.	.	.	.	1	1	4	3	.	.	.	.	.	.	.	.
F_1	.	.	.	.	.	.	.	.	.	.	.	.	4	1	.	.
F_2 651	.	.	.	.	1	1	3	1	.	.	.	.	1	1	.	.
653	.	.	.	.	1	1	3	1	.	.	.	.	1	6	1	.
654	.	.	1	.	.	.	1	.	.	.	.	1	1	2	2	1
655	1	.	.	1	.	.	2	1	.	1	.	4	7	3	1	.
656	.	1	1	.	1	1	1	1	1	.	1	3	3	7	1	.
657	.	1	1	.	2	2	4	1	1	1	4	7	8	10	1	4
658	.	1	.	1	.	.	1	.	.	.	1	1	.	2	.	.
659	1	1	.	.	.	1	3	.	1	1	.	1	4	10	2	1
Total	2	4	3	2	4	5	15	4	3	3	6	17	25	41	8	6

Full spot and intermediate spot are allelomorphic. There is some indication of bimodality in the recessive class, which may mean the segregation of a modifier.

The results do not indicate with certainty whether intermediate spot is a new member of the multiple allelomorphic series or is S^o, S^e, or S^w raised by modifiers. Crossing with full spot, however, did not raise S^e to more than grade 4, nor S^w to more than grade 9, so it is at least possible that Grenadines White Pollen is another member of the multiple allelomorph series.

7. Grenadines Naked (full) × Jamaica Long Staple (faint).

From the results in Table IX, it is seen that full spot and faint spot are allelomorphic, and that faint may rise, by introduction of modifiers, as high as grade 9. There is an indication of bimodality in the recessive class.

TABLE IX.

Results of the cross Grenadines Naked × Jamaica Long Staple.

Grade of spot

	0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
JLS	1	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
GN	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	5	5	1	.	.	.
F₁	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	3	.	.	.	.	.
F₂ 619	7	8	2	16	11	18	10	5	2	.	3	3	.	2	.	3	8	26	26	56	54	36	14	5
620	.	1	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	5	2	2	1
621	.	3	1	2	4	4	2	1	1	1	2	.	.	1	.	.	5	5	12	19	13	8	2	.
623	1	1	.	1	2	3	4	.	.	2	2	.	.	1	.	.	2	4	1	4	6	8	.	1
624	2	1	.	1	1	2	1	.	1	.	.	.	.	1	.	.	.	3	1	6	5	3	1	1
625	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	3	1
626	1	1	.	.	1	1	.	.	.	.	1	.	.	.	.	.	.	2	1	2	7	7	6	1
627 A	1	.	.	1	.	1	.	.	.	.	.	5	.	.	.	.	.	1	2	9	4	1	2	.
627 B	.	.	.	.	3	.	.	1	.	.	.	.	.	.	.	.	.	.	1	2	.	.	2	.
628	.	.	1	2	1	.	.	1	.	1	.	.	.	.	.	.	.	1	4	6	7	2	1	.
629	2	3	.	.	5	2	4	.	1	.	2	.	1	.	.	3	5	7	13	26	12	6	2	.
631	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1	1	2	.	.	.	.
632	.	1	.	.	.	.	.	.	1	.	.	.	.	.	.	2	1	2	4	2	1	.	.	.
Total	14	19	5	23	27	35	22	7	6	5	10	8	1	5	.	8	24	52	68	144	111	73	29	8

8. Trinidad Red Kidney (weak) × Jamaica Long Staple (faint).

In both these types spot is, in single flowers, frequently extinguished by fluctuation. The results (Table X) show that these two types constitute an allelomorphic pair of characters, for the gene **R** introduced by the Red Kidney parent shows the coupling type of linkage. The faint spot of *JLS* is thus either another member of the multiple allelomorphic series or is **S⁰**, or **Sᵉ**, raised by modifiers.

TABLE X.

Results of the cross Trinidad Red Kidney × Jamaica Long Staple.

Grade of spot

Family		0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12	13	14
JLS		1	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TRK		.	.	.	1	.	1	22	9	6	3	.	.	.	.	.	.	.
F₁																		
F₂	R	8	20	29	44	51	25	20	10	3	4	1	.	1	.	.	.	.
	r	34	19	4	6	2	.	1	3	.	1	.	.	.	.	.	.	1

9. Upland (spotless) × Sea Island White Flower (intermediate).

Sea Island White Flower is a mutant from the ordinary yellow form, and shows also a reduction in the grade of spot. Sea Island yellow extends up to grade 20, while Sea Island White does not extend above 14. It will be shown later that the reduced spot is a manifestation of the

recessive of the gene **Y** (yellow corolla). The results of this cross indicate greater complexity than those previously dealt with.

The following are the chief features of the results, which are seen in Table XI.

Spot varies in a continuous series from 0 to 19, *i.e.* higher than the Sea Island parent with modes at 12 (parental mode) and 17. There is a distinct point of minimum frequency at 7, and if this class is left out the ratio of spot above 7 to spot below 7 is not far from 9 : 7 :

	Above 7	Below 7
	295	195
Expected	275·4	214·5

TABLE XI.

Results of the cross Upland × Sea Island White Flower.

													Grade of spot											
Family	0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Upland	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SĪWF	.	.	.	.	.	.	.	.	.	.	.	3	5	10	32	4	.	.	.	.	.	.	.	.
F₁	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.
F₂ 547	16	4	.	1	.	1	.	.	.	.	.	3	1	3	6	7	1	3	4	4	1	.	.	.
548	35	4	5	4	3	4	.	3	.	.	8	7	6	14	28	19	8	9	6	18	3	5	.	.
549	52	5	4	7	2	6	5	1	4	1	7	5	10	17	14	7	8	8	9	8	4	.	.	.
550	2	1	.	.	.	.	.	.	1	1	.	.	.	1	1	.	.	.	.	.	.	.	.	.
552	7	.	1	.	1	.	.	1	.	2	.	.	1	1	4	2	2	2	3	2	.	.	.	.
553	5	.	1	.	.	.	.	1	.	1	.	1	1	3	3	2	2	2	.	1	.	.	.	.
Total	117	14	11	12	6	11	5	6	5	5	15	16	19	39	56	37	21	24	22	33	8	5	.	

Linkage between two complementary factors for high spot on a 2 : 1 : 1 : 2 basis would give a much closer fit :

	Above 7	Below 7
	295	195
Expected	299·6	190·4

There is, however, no experimental evidence of this linkage in this cross and speculation is thus somewhat idle. As the gene for red is missing from the parents, there is no definite line of demarcation between **S**s and its allelomorph, so that no factorial analysis can be made. It is, however, certain that there is not simple unifactorial segregation.

It is interesting to note that if the ratio of spotless to the rest be taken, it comes out almost exactly to a 3 : 1 ratio: 373 : 117 (expectation 367·5 : 122·5). This probably means very little, as it has been pointed out in the discussion of previous crosses that modifying genes produce a ratio in all cases, of more spot to less spot, and never of spotted to absolutely spotless.

TABLE XII.

Results of the Selfed Back-Cross (Upland × Sea Island) × Sea Island.

1. Homozygous **Y** and heterozygous **S**.

Grade of spot

Family	0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
429	1	.	.	1	.	1	1	.	.	.	.	.	.	1	3	2	1	1	.	2	.	1	.
432	1	1	2	4	1	2	.	.	.	.	.	1	2	2	6	4	3	3	1	5	3	.	.
433	1	.	.	2	.	1	.	.	.	.	.	.	.	1	1	.	1	2	2	.	.	.	1
434	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.
459	1	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	1	3	.	1	.	.
461	1	1	2	.	1	.	.	.	.	1	1	.	.	2	4	1	4	4	2	1	.	1	.
483	1	1	.	1	.	.	.	.	.	.	.	.	1	.	1	.	.	.	2	.	.	.	.
Total	6	3	4	8	3	4	1	.	.	1	1	1	3	6	15	8	8	10	10	10	5	2	1

2. Homozygous **Y** and homozygous **S**.

Grade of spot

Family	10	11	12	13	14	15	16	17	18	19	20	21
444	.	.	3	3	2	8	6	3	2	.	1	1
463	.	.	.	.	1	.	.	4	.	.	.	.
466	1	.	.	.	.	.	.	2	2	.	.	.
473	.	.	2	.	.	.	.	2	1	.	.	.
474	.	.	.	.	.	1	1	8	.	2	.	2
478	.	.	.	1	1	1	1	1	1	.	.	.
487	.	1	1	.	1	1	2	1	4	.	.	.
493	.	.	.	1	1	1	.	4	2	.	2	4
Total	1	1	6	5	6	12	10	25	12	2	3	7

3. Heterozygous **Y** and heterozygous **S**.

Grade of spot

Family	0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
423	1	1	.	.	2	.	.	.	.	.	.	.	.	1	3	.	1	1	2	.	2	2	.	.
424	7	1	3	2	1	1	2	2	.	1	3	2	2	2	8	4	3	1	1	7	2	.	.	.
430	3	1	.	.	1	.	2	.	3	2	3	1	5	2	8	2	6	5	5	4	2	.	.	.
436	.	.	1	2	.	1	2	.	1	.	1	.	2	3	4	.	3	2	3	4	2	.	.	.
437	.	.	.	2	.	1	.	1	.	1	.	.	.	1	3	2	.	5	7	7	2	1	1	1
441	.	1	2	1	1	1	1	.	.	.	1	.	1	2	6	1	3	1	3	2	.	.	.	.
446	1	3	2	1	.	2	.	.	1	.	.	.	.	.	3	1	3	2	1	7	5	.	.	.
468	.	.	.	.	.	.	.	.	.	.	.	1	1	.	.	1	1	.	.	.	.	.	.	.
486	.	1	.	.	.	.	.	.	.	.	.	.	.	2	2	.	1	.	.	.	.	.	.	.
Total	12	8	8	6	8	5	8	2	5	4	8	4	11	13	37	11	21	17	22	31	15	3	1	1

4. Heterozygous **Y**. Homozygous **S**.

Grade of spot

Family	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
439	.	.	.	.	1	2	2	1	1	3	1	4	2	1	.
440	.	1	.	.	.	2	.	.	.	.	6	2	5	1	.
443	.	.	.	.	.	.	1	.	3	3	8	6	13	7	10
460	.	.	.	.	.	.	.	1	1	1	.	2	.	.	.
482	.	.	.	.	.	.	.	.	.	.	1	1	.	.	.
Total	.	1	.	.	1	4	3	2	5	7	16	15	20	9	10

10. (Upland × Sea Island) × Sea Island, selfed.

A large number of families have been studied of a selfed back-cross of the above type, and the results obtained will bear detailed analysis.

The complete results are presented in Table XII.

It will be seen that separate consideration has been given to families homozygous and heterozygous for **Y** (yellow corolla). It was observed that in some families, clearer segregation into strong spot and weak spot took place than in others, and it was further found that this was so in families homozygous for yellow corolla (**Y**). In families segregating for **Y** the point of minimum frequency was grade 7. Taking out grade 7, the ratio of strong spot to weak spot is as follows:

	Spot strong	Spot weak
Families homozygous **Y**	81	29
Expected	82·5	27·5
Families heterozygous **Y**... ...	195	62
Expected	192·75	64·25

There is here quite definite evidence that strong spot and weak spot are segregating in a simple 3 : 1 ratio; but whereas in families homozygous for **Y** intermediate grades are practically missing, the families heterozygous for **Y** show the same type of segregation, more or less obscured by intermediate grades. In this type of family, indeed, there is no real discontinuity between strong and weak spot. The separation has been effected at grade 7 partly because it is the point of minimum frequency in three of the segregating families, and partly because it was clearly the point of minimum frequency in the previous cross Upland × Sea Island White. The presence of intermediate grades in heterozygous **Y** families, and not, as a rule, in homozygous **Y** families, is due to the fact that **Y** is linked with a spot modifier. The consequence of this is that plants homozygous for yellow are mostly homozygous for the spot modifier. If families with the intermediate grades filled in are accepted as segregating for the modifier the families can be divided thus:

	Homozygous **Y**		Heterozygous **Y**	
	Hom. modifier	Het. modifier	Hom. modifier	Het. modifier
Families	429	461	423	424
	432	.	468 or 486	430
	433	.	.	436
	434	.	.	437
	459	.	.	441
	483	.	.	446
Total	6	1	2	6

It may be surmised that **Y** and the modifying gene which fills in the

intermediate grades of spot from 4 or 5 to 7 or 8 are about 20 units apart on the same chromosome.

The nature of the modifier. Having gained some idea of the action of the modifier in families segregating for the main spot factor, the distribution of grades of spot in homozygous spot families may be discussed. If in **SS** families heterozygous for **Y** the intermediate grades are filled in, such families should show a greater upward range for spot than **SSYY** families. The distribution of spot grade in the two types of family is shown in diagram 1, from which it will be seen that there is

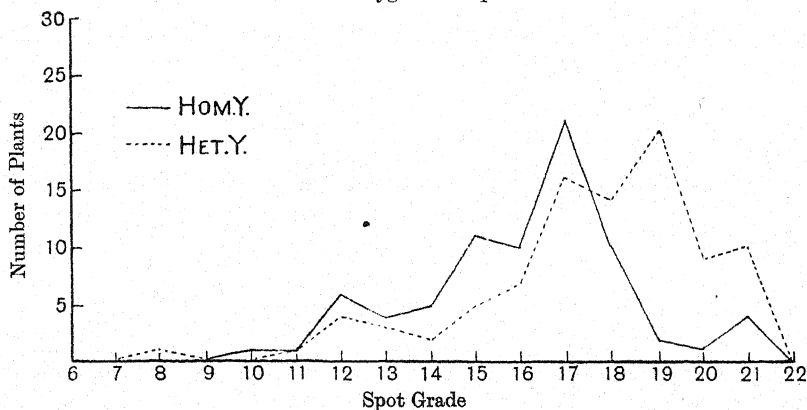Variation in Spot Grade in Families
Homozygous for Spot.

Diagram 1.

a mode at grade 19 in families heterozygous for **Y** and at 17 in families homozygous for **Y**, i.e. the modifier is of the nature of a dominant inhibitor of the main spot gene. The grades in the four terms of the dihybrid ratio may be pictured as follows:

			Grade
SY with Inhibitor	...	...	17
SY no Inhibitor	...	...	19
Sy with Inhibitor	...	...	4
Sy no Inhibitor	...	...	6

These figures are naturally approximate.

Linkage between spot and **Y**. Regarding grade 7 as the point of minimum frequency and plotting separate frequency arrays for spot for **Y** and **y**, the following result is obtained:

	YS	**Ys**	**yS**	**ys**
	160	29	25	32
Expected on 3 : 1 : 1 : 3 basis ...	157·6	26·9	25	34·6

The percentage of crossing-over is 25.

Linkage between **Y** *and spot modifier.* The frequency arrays for **Y** and **y** in families *homozygous* for spot show that **Y** and the spot modifier are linked, though on what basis is not easy to determine. The frequency arrays are found below:

	Grade of Spot													
	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Y	1	.	.	1	2	4	9	8	7	23	17	51	14	13
y	.	1	.	2	4	3	2	1	2	7	5	5	2	1

Here it will be observed that whereas in grades up to 14 the ratio of strong spot to weak spot is 17 : 12, the ratio in grades 19, 20 and 21 is 78 : 8, indicating linkage of **Y** and the spot modifier. If all above 15 be called high spot, and all below low spot, the numbers become:

	Y high	Y low	y high	y low
	100	23	22	15
Expected on 2 : 1 : 1 : 2 basis ...	97·8	22·2	22·2	17·8

On the basis of these numbers the percentage of crossing-over is approximately 33.

Relation of **Y** *and* **S** *in crosses involving* **R**. It has been shown that in Upland × Sea Island crosses **Y** is linked with a gene for strong spot. Examination of all the previous crosses involving both **R**, **Y**, and spot show that no linkage exists between **R** and **Y**, or between **Y** and spot. The following examples will suffice to prove this point.

1. F_2 Red Kidney × Upland.

	Grade of Spot													
	0	·25	·50	1	2	3	4	5	6	7	8	9	10	11	12	13
Y	353	111	21	38	16	16	8	4	6	4	10	.	5	1	2	1
y	129	21	5	5	11	5	.	1	1	2	1	.	2	.	.	.

It is clear that no correlation exists here between **Y** and spot.

2. Upland **ys** × (10 B 27 × *TRL*) **YSys**.

	YS	Ys	yS	ys
	285	329	276	362
Expected	313	313	313	313

Here the deviation from the expected 1 : 1 : 1 : 1 ratio is fairly large, probably due either to elimination of certain zygotic combinations or to differential pollen tube growth, but there is no evidence that **Y** and spot are linked.

It will be shown in a subsequent paper that **Y** and **R** are independent in inheritance. Hence the particular spot genes in the Upland × Sea Island × Sea Island material are not the same as those previously demonstrated

to be linked with **R**, and until **R** is brought into the Upland-Sea Island combination the relation between spot linked with **R** and spot linked with **Y** will not be determined.

SUMMARY.

The main points demonstrated in this paper on the mode of inheritance of various types of petal spot in cotton may be summarised thus:

1. Weak spot of *TRK*, S^w, is allelomorphic to spotless Upland, S^o, and linkage exists between S^w and **R** (red).

2. Extinguished spot of Cassava, S^e, is allelomorphic to spotless Upland, S^o, and linkage exists between S^e and **R**.

3. Full spot of Pima Egyptian, S^s, is allelomorphic to extinguished spot of Cassava, S^e, and linkage exists between S^s and **R**.

4. Full spot of Sea Island, S^s, is allelomorphic to weak spot of *TRK*, S^w, and linkage exists between S^s and **R**.

5. Switching over of the linkage to a repulsion type indicates that full spot, weak spot (and or extinguished spot) and spotless form a system of multiple allelomorphs.

6. Red and spot of the multiple allelomorph series are linked with 4·3 per cent. of cross-overs.

7. Full spot of Pima Egyptian, S^s, is allelomorphic to intermediate spot of Grenadines White Pollen, S^i.

8. Full spot of Grenadines Naked, S^s, is allelomorphic to faint spot, S^f, of Jamaica Long Staple.

9. Weak spot of Trinidad Red Kidney, S^w, is allelomorphic to faint spot of Jamaica Long Staple, S^f, since **R** and weak spot are linked.

10. Strong spot, above grade 7, in Upland × Sea Island White may be due to two complementary linked genes, but the evidence is not complete.

11. The main gene for strong spot in Upland × Sea Island × Sea Island is linked with **Y** (yellow corolla) with 25 per cent. crossing-over.

12. In this cross linkage also exists between **Y** and a spot modifier with 20 to 33 per cent. crossing-over.

13. The spot gene linked with **Y** is not the same as the spot gene linked with **R**.

14. Modifying factors may profoundly affect the development of spot in a plus or minus direction.

GENERAL REMARKS.

The results obtained and described in this paper show how a genetic difference can be affected by modifying genes, so as to show sharp segregation, or to bring the dominant so close to the recessive in appearance that it becomes indistinguishable from it. It is clear, for example, that extinguished spot is allelomorphic to a lower grade of spot, although both are normally invisible.

Sharpness of segregation between dominant and recessive is thus found (a) when the dominant gene can be clearly distinguished from its allelomorph, in the presence of all combinations of modifying factors; (b) when a favourable constellation of modifiers is present in both dominant and recessive.

Condition (b) is fulfilled when an ordinary loss mutation occurs, for it results in only a single genic difference, i.e. affects only one locus in the chromosome.

The situation may be represented thus:

SABCDE   Loss mutation in the spot locus. No segregation in other modifying genes.
sABCDE   Result is clear segregation 3 full spot : 1 weak spot.
SAbcDE   An interspecific cross. Segregation also for four modifiers. Produces a range
sabCde   of types of spot which may defy classification, from intermediate spot to
      spotless.
Sabcde   Spot not visible at all, owing to unfavourable genic balance of modifiers.
sabcde   Segregates for the dominant gene nevertheless.

The rule that the difference between a dominant and a recessive may be reduced, almost if not quite, to vanishing point by modifying factors has been found to hold good not only for spot on the petal, but also for the characters Pollen colour, Corolla colour, and Red leaf. Details will appear in subsequent papers.

The origin of new recessive mutants sharply distinguished from the normal type and showing clear segregation into 3 dominant and 1 recessive has been frequently observed in both plants and animals. New dominants, however, of normal viability have not been observed to arise. This is now capable of a simple explanation. For a new dominant to arise it means that a favourable constellation of modifiers must be present in the type, and no mechanism can at present be imagined which will put all the modifying genes in their right proportions ready for the dominant to appear. Let us imagine a jig-saw puzzle, with one unit changed. Here the picture without the unit or with the unit changed can be taken to represent the recessive mutant, i.e. you have two things —the picture, and the picture with one unit changed. To get a dominant

mutation you would have to have your jig-saw puzzle with the units all complete, set in their right places, and simply fill in the missing unit. It will be seen that the chance of this happening is remote.

It is not without significance that in plants which have been studied most successfully from the genetic point of view, and in which the characters segregate sharply, *e.g.* the Sweet Pea—that in course of time many large (*ausgiebige*) mutations have arisen, which segregate sharply from the normal type. Each of these mutants is a single genic difference in a ready-made genetic background, and that is why sharp segregation occurs. If, however, the Sweet Pea (*Lathyrus odoratus*) were crossed with another species altogether, say *Lathyrus hirsutus*, it is improbable that any of these mutations would be sharply inherited, simply because the genetic background in the latter species would undoubtedly be different, and segregation would occur, not only in the main gene but in a large number of other genes which modify its expression. A range of types would often be found grading from the mutant to normal.

The question why sharp non-lethal dominant mutations do not occur may be partly answered thus:

1. Dominance is often purely a question of genic balance (like sex in *Drosophila*).

2. Potential dominants probably exist in large numbers in the plant already but have only a small effect because of unfavourable genic balance, like extinguished spot in cotton.

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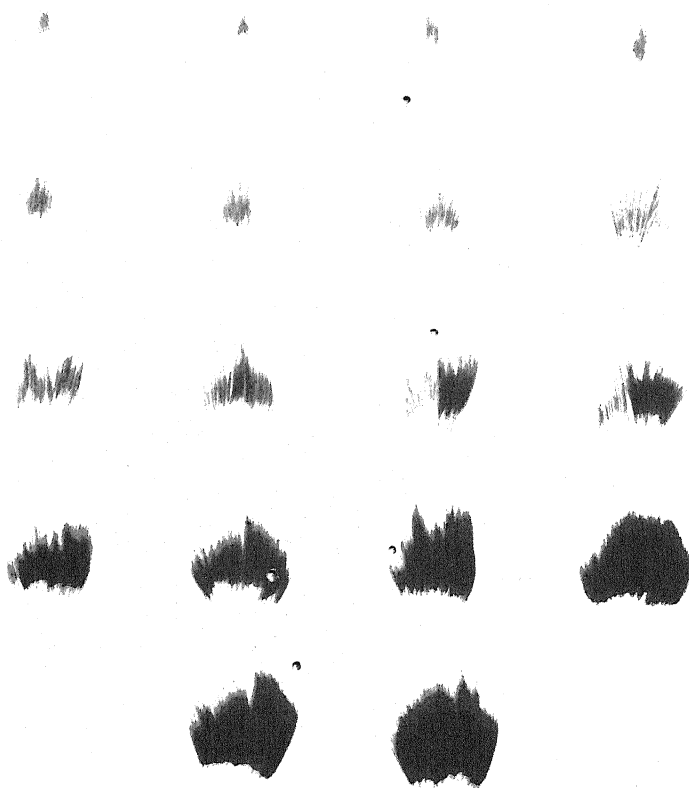
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EXPLANATION OF PLATE IX.

These coloured drawings indicate the 22 grades of petal spot in cotton of which use was made during the investigation. For further explanation see text, p. 368.

THE GENETICS OF COTTON. PART II. THE INHERITANCE OF POLLEN COLOUR IN NEW WORLD COTTONS.

By SYDNEY CROSS HARLAND.

(With One Colour Plate.)

CONTENTS.

INTRODUCTION.

THE colour of the pollen in New World cottons varies from pale cream to rich golden yellow. In Old World cottons deep yellow is the usual colour though paler shades have been noted. Pollen of a golden yellow colour is characteristic of many other Malvaceous plants belonging to related genera, e.g. Thespesia and Hibiscus. In the cultivated Hibiscus rosa-sinensis a range of grades from pale cream to deep yellow is also found.

PREVIOUS INVESTIGATIONS.

Balls (1912) studied the inheritance of anther colour, which is inseparable from pollen colour. He worked with Egyptian-Upland crosses. Upland is buff, Egyptian is golden yellow. He claimed F_1 intermediate, and F_2 segregating into a normal $1 : 2 : 1$ ratio. A family of King Upland, the parent of which was pale lemon, gave 24 lemon and 8 buff.

McLendon (1912) states that in Upland-Sea Island crosses yellow by buff gave intermediate. In F_2 the expected simple Mendelian ratio was not obtained, but the parental forms reappeared in varying proportions in different crosses with continuous variation between the extremes. He states further that absolute correlation exists between petal colour and anther colour, but this has been observed by no other worker.

His ratios vary from 12·5 : 1·0, to 4·1 : 1·0, showing a deficiency of the Upland shade.

Kearney (1923) using Ridgeway's colour scale crossed Upland (pale chalcedony yellow) × Egyptian (Empire yellow). F_1 gave partial dominance of the yellow colour of Pima. Some indication of a bimodal distribution was shown by the F_2, the pale colour appearing to be a simple recessive. Kearney's results thus fall into line with those of Balls.

GRADES OF POLLEN COLOUR.

At the outset of the experiments it was seen that a more accurate system of grading than that of previous investigators would have to be devised. Accordingly nine grades were established, and painted accurately in water-colours for reference. The paintings were frequently compared with the original types. The grades, which are reproduced on Pl. X, were as follows:

Grade	Type	Group
0	Upland, Grenadines White Pollen	Upland
0·5	Upland (0) × Bourbon Intense (1) F_1	—
1·0	Trinidad Red Leaf	Bourbon
1·5	Punjab Golden	Upland
2·0	F_1 Meade × Egyptian Pima	—
2·5	Jamaica Long Staple, Sea Island Naked (*SIN*)	Peruvian
3·0	Egyptian Pima	,,
3·5	Egyptian	,,
4·0	Caravonica	,,

Grade 0. The standard type is a variety of Upland—Superokra. Grade 0 has not been systematically subdivided but consistent variations in shade have been observed. The highest grade of 0 is found in the Peruvian type Grenadines White Pollen (G.W.P.) which comes between the standard grade and the next grade 0·5. The pollen colour of the Upland variety Meade, used in our experiments, is slightly deeper in shade than standard 0, but not so deep as that of G.W.P. Variations in grade 0 are accompanied by corresponding variations in the F_1 of crosses between 0 and 3. Thus, the cross *SIN* (2·5) by Superokra (standard 0) produces an F_1 of a grade slightly below that of *SIN* by Meade. Grade 0 is also found in the native Trinidad variety of Bourbon known as Volunteer.

Grade 0·5. The standard is Meade (0) × Bourbon intense (1), but examples are also met with in the F_2 of crosses between the Bourbon variety Cassava, and Upland.

Grade 1. The standard is the Bourbon type, Trinidad Red Leaf, but many Bourbons (Cassava, Bourbon intense, etc.) are of this shade, which has not been seen so far in the Peruvian group.

Grade 1·5. The standard is a variety of Upland known as Punjab golden, a selection from the Punjab American variety 285 *F*, which apparently consists of a mixture of yellow and white pollened plants. This shade has been seen in many commercial Upland varieties, and also in native cottons of Upland type from Guatemala.

Grade 2. Grade 2 is the heterozygote between Pima Egyptian (3) and Meade (0). The Egyptian variety Enan's Brown is apparently a homozygous type of this grade, but a sufficiently large number of plants has not yet been examined. Slight variations in this grade occur due to differences (*a*) in the type of grade 0 used in making the cross, and (*b*) when Sea Island (2·5) is used to make the cross instead of Pima a grade 2 F_1 is produced slightly below the standard grade.

Grade 2·5. The standard type is Sea Island Naked, but all Sea Island varieties so far examined belong to this grade, as well as the Peruvian types Jamaica Long Staple, Cauto and Trinidad Red Kidney.

Grade 3. The standard type is a pure line of Egyptian Pima.

Grade 3·5. This grade was made from an Egyptian plant of unknown parentage, which has since been lost. It has not been used in the present experiments.

Grade 4. The standard is the Peruvian variety Caravonica, obtained from Hawaii. This variety has not entered into the experiments. Grades 2·5 to 4 are so far known only in the Peruvian group of New World cottons.

Grades in the wild species of Gossypium. Of the so-called wild species, *G. Stocksii* has grade 0 pollen, *G. Sturtii* grade 0 pollen occasionally tinged with anthocyanin, *G. Davidsonii* grade 1 and *G. tomentosum* grade 3.

SUMMARY OF THE PRESENT RESULTS.

1. A single genetic difference is involved in all crosses between any grade of yellow and grade 0. That is, yellow and pale cream form a simple pair of factors, which may be denoted as **P** and **p**.

2. Modifying genes act on the basal gene for yellow, producing a complex series of shades from pale yellow 0·5 to deep golden 4·0. These modifying genes can be carried by grade 0, producing such minute variations in shade as are exemplified by Superokra and G.W.P.

3. **P** produces little effect when unsupported by modifiers, and the distinction between **P** and **p** is often hard to make in segregating families lacking such modifiers. It is possible that in the absence of any modifier at all, the distinction between **P** and **p** would vanish,

i.e. **P** would produce too pale a shade of yellow to observe segregation.

4. In crosses between cottons of the same class, *e.g.* inter-Peruvian or inter-Upland, segregation is usually very sharp, owing to similar modifying factors being present in a homozygous condition in both parents.

5. In the selfed back-cross (Upland × Sea Island) × Sea Island, an intensifier, **Q**, was demonstrated, having no visible effect except in presence of **P**.

<div align="center">THE EXPERIMENTAL DATA.</div>

Segregation of yellow (**P**) *and cream* (**p**).

The ratio of **P**—all grades above 0, to **p**—grade 0, has been worked out for all the segregating families and in back-crosses and presented in Table I. The results do not demand comment, as they establish clearly

<div align="center">TABLE I.</div>

A. *Segregation of families into yellow* (**P**) *and cream* (**p**).

Series	Yellow (**P**)	Cream (**p**)
1	120	39
2	798	267
3	684	248
4	369	123
5	536	157
7	338	110
8	308	104
Total	3153	1048
Expected	3150·75	1050·25

B. *Segregation in back-crosses of* **Pp** × **pp**.

Series	Yellow (**P**)	Cream (**p**)
1	816	881
2	637	811
3	317	365
Total	1770	2057
Expected	1913·5	1913·5

that the basal factor **P** producing some shade of yellow is allelomorphic to cream (grade 0). In back-crosses there is a deficiency of **p**, the reason for which will form the subject of a later communication. Grade 0 always breeds true in F_3 families or in selfed back-crosses. This statement is based on the results of many thousands of plants and it is unnecessary to present detailed figures.

Segregation of shades of yellow in different crosses.

The results of crosses between various grades of yellow and cream will now be described, and of one case of a cross between two grades of yellow.

1. The inter-Peruvian cross, Pima Egyptian (3) × Grenadines White Pollen (0).

Pima is a well-known variety of Egyptian isolated by Kearney in Arizona. Grenadines White Pollen (G.W.P.) is a variety of the Peruvian type of West Indian perennial known as Marie Galante, and, cultivated as a pedigree selection, it bred true.

The F_1 is grade 2·5, almost complete dominance being shown.

TABLE II.

Results of the cross Pima (3) × Grenadines White Pollen (0).

Family	Pollen grade					
	0	1·0	1·5	2·0	2·5	3·0
Pima	.	.	.	.	7[1]	5
GWP	10	.	.	.	.	.
F_1	.	.	.	.	6	.
F_2 651	.	.	.	.	2	.
653	4	.	.	.	6	3
654	2	.	.	.	6	4
655	5	.	.	.	11	6
656	8	.	.	2	8	2
657	11	.	.	4	31	7
658	0	.	.	.	6	1
659	9	.	.	2	15	4
Total	39	.	.	8	85	27

The F_2 results are shown in Table II. Segregation is sharp, and a 3 : 1 ratio of yellow to pale cream is observed:

	Yellow (**P**)	Cream (**p**)
Observed	120	39
Expected	119·25	39·75

2. The inter-Upland cross Acala (0) × Punjab Golden (1·5).

The F_1 is intermediate—grade 1.

The F_2 results will be found in Table III. All plants were examined

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[1] The examination of the grades was made early in the season of 1927 when 2·5 could not be distinguished with ease from 3·0. Later examinations of other Pima families have invariably given nothing but 3·0. The distinction between 2·5 and 3·0 made in the F_2 table is similarly not absolutely to be relied on. That is, some plants classified as 2·5 are probably 3·0. The plants classified as 2·0 must be placed in grade 2 as they are lower than 2·5, but are a little above our standard 2.

TABLE III.

Results of the cross Acala × Punjab Golden.

Family	Pollen grade					
	0	1·0	1·5	2·0	2·5	3·0
Acala	*	.	.	.	.	.
Punjab Golden	.	.	*	.	.	.
F_1	.	*	.	.	.	.
F_2 501	24	12	25	15	.	.
502	26	4	33	22	.	.
Total	50	16	58	37	.	.

for **P** and **p**, while one family was picked out for detailed examination of grades. The numerical results for **P** and **p** are placed below:

	Yellow (**P**)	Cream (**p**)
Obtained	798	267
Expected	798·75	266·25

The grade of yellow in F_2 ranges from 1 to 2^1.

3. The Upland-Peruvian cross, Upland (0) × Trinidad Red Kidney (*TRK*) (2·5).

The F_1 is intermediate—grade 2 (standard).

TABLE IV.

Results of the cross Upland × Trinidad Red Kidney.

Family	Pollen grade					
	0	1·0	1·5	2·0	2·5	3·0
Upland	*	.	.	.	.	.
TRK	.	.	.	.	*	.
F_1	.	.	.	*	.	.
F_2 555	40	1	9	38	9	47
556	52	.	5	17	17	57
557	8	.	2	7	1	9
558	66	.	27	46	23	72
559	65	2	30	77	37	96
560	17	.	7	21	10	17
Total	248	3	80	206	97	298

The F_2 results are found in Table IV. The grade of yellow ranges from 1 to 3, but grade 1 is very rare—only 3 out of 684. Modes exist at grades 2 and 3.

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[1] Shrivelled pollen grains were encountered very frequently in the F_2 plants, and as these pollen grains are deeper in colour than normal ones the classification of types put in grade 2 is not entirely satisfactory. It is believed, however, that many of the grade 2's are valid, and if they are correctly graded it implies that modifying factors have been brought into the cross from Upland.

The numerical results for **P** and **p** are placed below:

			Yellow (**P**)	Cream (**p**)
Obtained	...	...	684	248
Expected	...	...	699	233

4. The Upland-Peruvian cross, Upland (0) × Sea Island White (2·5).

The F_1 is grade 2, as in the last cross, but slightly lower than the standard.

In the F_2 the yellows range from 1·5 to 4, but there are only 3 plants above grade 3 out of 269. The pollen colour in these plants, is definitely more intense than grade 3. The proportion of grade 1·5 is less in this cross than in No. 3. The results are presented in Table V.

TABLE V.

Results of the cross Upland × Sea Island White Flower.

					Pollen grade				
Family	0	1·0	1·5	2·0	2·5	3·0	3·5	4·0
Upland	*		.	.	.	.	.	.
SI WF	.	.	.	.	*	.	.	.
F_1	.	.	.	* .	.	.	.	.
F_2 547	15	.	1	17	9	19	.	.
548	47	.	5	48	18	64	.	1
549	49	.	13	64	20	44	1	1
550	2	.	.	3	1	.	.	.
552	5	.	1	13	1	8	.	.
553	5	.	2	7	1	7	.	.
Total	123	.	22	152	50	142	1	2

The numerical results for **P** and **p** are set out below:

			Yellow (**P**)	Cream (**p**)
Obtained	...	...	369	123
Expected	...	...	369	123

5. The Upland-Bourbon cross, Upland (0) × Cassava (1).

The F_1 is grade 1, showing complete dominance of yellow.

The F_2 shows a range in the yellow class from 0·5, a grade not previously recorded, to grade 2, thus transcending the limits of the parents in both directions.

Classification in this cross is complicated considerably by segregation in the time of opening of the anthers. All Bourbon cottons are "late bursters" and do not shed their pollen often till about 10.30 a.m. as against the early bursting—6 a.m. to 7 a.m. of Uplands and Peruvians. The colour of the pollen gradually darkens as it becomes older, and our grades are based on the colour at the time of opening. Thus, grade 1 becomes 1·5 by afternoon, while the higher grades show relatively little

change. For this reason it is important to grade every plant at the time of bursting but it has not been possible to do this in every case, as examination consumes an appreciable time. In this case grading has been done using flowers the grades of which were determined earlier in the morning by the chart and which are assumed to have darkened at the same rate as those under examination.

TABLE VI.

Results of the cross Upland × Cassava.

Family	Pollen grade				
	0	0·5	1·0	1·5	2·0
Upland	*	.	.	.	.
Cassava	.	.	*	.	.
F_1	.	.	*	.	.
F_2 414	84	11	107	169	16
416	34	25	59	42	2
417	15	5	33	50	.
418	37	8	23	54	2
419	26	5	37	35	.
420	39	7	46	52	.
Total	235	61	305	402	20

Three families show no plants of grade 2. The results are presented in Table VI. The numerical results for **P** and **p** are placed below:

			Yellow (**P**)	Cream (**p**)
Obtained	...	...	788	235
Expected	...	...	767·25	255·75

6. The Peruvian-Bourbon cross, Pima Egyptian (3) × Cassava (1).

The F_1 is grade 1·5, in one plant and 2 in another.

The F_2 shows a range from 1 to 3 with modes at 1·5 and 3, grade 1 occurring in 4 plants out of 50. This might indicate a bigenic difference

TABLE VII.

Results of the cross Pima × Cassava.

Family	Pollen grade							
	0	1·0	1·5	2·0	2·5	3·0	3·5	4·0
Pima	.	.	.	.	.	*	.	.
Cassava	.	*	.	.	.	.	.	.
F_1	.	.	1	1	.	.	.	.
F_2 677	.	.	2	1	.	2	.	.
678	.	1	2	.	.	1	.	.
679	.	1	6	4	1	5	.	1
680	.	1	2	.	.	.	.	.
681	.	.	2	.	.	.	.	.
684	.	1	6	1	3	7	.	.
Total	.	4	20	6	4	15	.	1

between grade 1 and grade 3. Two plants occurred with pollen of a reddish tinge, which did not exactly fit any of the grades, but was nearest to 3. The results, which do not permit of a factorial analysis, are to be found in Table VII.

7. Plant 2 (Upland × Egyptian) × Upland (**Pp**) (Grade 2).

In addition to the above crosses, which have been carried only as far as F_2, a somewhat detailed study has been made of the descendants of a single plant known as plant 2, an Upland × Egyptian F_1 heterozygous for **P**. It was back-crossed with Upland and detailed observations on pollen colour have been carried on for two or more generations, the results of which are given in Table VIII.

TABLE VIII.

Pollen colour in descendants of Plant 2 (Upland × Egyptian) × Upland.
*Grade 2, **Pp**.*

Family	Parent grade	Pollen grade					
		0	1·0	1·5	2·0	2·5	3·0
2	2	110	20	123	107	23	65
2–8	1·5	.	.	6	3	.	.
2–14	2·5	.	.	3	9	10	11
2–15	1·5	16	.	16	6	2	5
2–16	2·0	2	.	.	3	1	.
2–20	1·5	2	.	.	3	6	.
2–28	1·5	10	.	6	6	5	1
2–30	3·0	.	.	.	.	1	4
2–32	.	31	.	11	23	1	37
,,	.	51	.	36	31	7	34
2–38	.	16	.	9	11	4	4
2–39	.	.	3	57	36	4	—
2–41	.	5	.	2	1	1	2
2–51	.	10	.	.	1	.	19
2–52	3·0	.	.	.	.	.	22
2–53	.	20	.	39	25	.	6
2–54	.	8	1	15	.	.	.

The main points of importance are as follows:

The large family of 448 plants, progeny of plant 2, shows segregation into the usual 3 : 1 ratio as regards the main factor **P**:

			Yellow (**P**)	Cream (**p**)
Observed	...	...	338	110
Expected	...	...	336	112

In this family grades of yellow were produced from 1 to 3, but the general type of segregation is different from that in crosses 1 and 3. There are proportionally many more plants of grades 1 and 1·5.

In the next generation segregation is less complex. One case is seen (2–52) of a homozygous grade 3, identical or nearly so with Egyptian.

Family 2–51 shows segregation into grade 3 and grade 0, with only a single plant of grade 2, possibly due to vicinism. This type of segregation is practically identical with that found in the inter-Peruvian cross, No. 1, Pima × Grenadines White Pollen. It is evident that the same modifying factors accompany both **P** and **p**.

It may be supposed that the yellows have one or more modifying genes, which convert all the paler shades of yellow to grade 3, and are present in the cream class with little visible effect. Families 2–14 and 2–39 provide examples of families breeding true to yellow but with dissimilar ranges, 2–14 ranging from 1·5 to 3·0, and 2–39 from 1·0 to 2·5. A type of segregation rather like that of the inter-Upland cross 2, is seen in family 2–54 where there is practically straight segregation into 1·5 and 0.

8. (Upland (0) × Sea Island (3–2·5)) × Sea Island (3–2·5).

A number of plants in the above cross were selfed, and the pollen colour in the resulting families carefully graded. At the time of grading accurate classification into 3 and 2·5 was not yet certain. The results are presented in Table IX and show the following points of interest:

Plants of grades 3 and 2·5 show the following types of behaviour:

  (a) breed true;
  (b) segregate into 3 and 2;
  (c) segregate into 3, 2·5, 2 and 1·5;
  (d) segregate into 3, 2·5 and 0 with practically no intermediates.

Plants of grade 2 produce all grades from 3 to 0.

These results may be accounted for on the basis of one main gene **P** and an intensifying gene with no visible effect except in presence of **P**. Calling the intensifier **Q** we have for the back cross:

$$\text{(PQ} \qquad \times \text{pq)} \qquad \times \text{PQ}$$
$$\text{(3} \qquad \qquad \text{0)} \qquad \quad \text{3}$$

giving four types of plants in equal numbers:

Type			Families	
			Obtained	Expected
1	**PQ** **PQ**	Breeding true to 3 and 2·5	13	13·5
2	**PQ** **Pq**	3 and 2·5 throwing lower grades of yellow	16	13·5
3	**PQ** **pQ**	3 and 2·5 throwing 0	14	13·5
4	**PQ** **pq**	2 throwing all grades from 3 to 0	11	13·5

TABLE IX.

Results of the selfed back-cross (Upland × Sea Island) × Sea Island.

Grades 3 and 2·5 breeding true to grade 3 and 2·5. 13 families.

Family	Parent	\<-- Pollen grade --> 0	0·5	1·0	1·5	2·0	2·5	3·0
430	3	.	.	.	.	.	4	64
446	3	.	.	.	.	.	1	56
459	3	.	.	.	.	.	.	7
464	3	.	.	.	.	.	.	7
478	3	.	.	.	.	.	.	12
487	.	.	.	.	.	.	4	8
496	.	.	.	.	.	1	5	17
502	.	.	.	.	.	1	1	39
514	?	.	.	.	.	.	.	12
517	?	.	.	.	.	.	.	7
535	3	.	.	.	.	.	.	10
588	3	.	.	.	.	.	2	11
584	.	.	.	.	.	.	.	6

Grades 3 and 2·5 segregating down to 2 and 1·5. 16 families.

Family	Parent	0	0·5	1·0	1·5	2·0	2·5	3·0
423	2·5	.	.	.	.	5	3	9
424	3	.	.	.	1	16	12	39
432	.	.	.	.	3	31	7	9
433	3	.	.	.	.	5	.	11
439	.	.	.	.	.	2	8	25
443	.	.	.	.	.	5	3	64
462	2·5	.	.	.	.	1	2	2
463	3	.	.	.	.	1	.	4
486	3	.	.	.	.	1	.	6
492	3	.	.	.	.	7	7	9
519	3	.	.	.	.	2	5	11
520	?	.	.	.	.	3	1	4
521	?	.	.	.	4	11	3	7
530	3	.	.	.	.	2	.	3
554	3	.	.	.	.	3	1	4
568	.	.	.	.	.	3	3	13

Grade 3 segregating down to 0. 13 families.

Family	Parent	0	0·5	1·0	1·5	2·0	2·5	3·0
437	.	8	.	.	.	7	6	24
441	.	12	.	.	.	8	5	16
461	2·5	2	.	.	.	6	.	16
468	2·5	4	.	.	.	.	1	4
473	2·5	1	.	.	.	.	.	4
477	3	1	.	.	.	1	.	5
495	.	1	.	.	.	.	.	8
497	.	4	.	.	1	2	.	9
500	.	3	.	.	.	1	1	8
504	.	2	.	.	.	4	1	10
511	3	5	.	.	1	3	1	10
515	3	2	.	.	.	1	2	2
528	.	2	.	.	.	.	.	4

Grade 2 segregating down from 3 to 0. 12 families.

Family	Parent	0	0·5	1·0	1·5	2·0	2·5	3·0
429	2	2	.	.	.	6	1	4
434	2	2	.	.	1	1	1	3
436	2	9	.	.	4	11	3	8
440	.	6	.	.	3	6	3	4
444	.	10	.	.	3	11	6	13
466	2	3	.	.	1	4	.	5
474	2	10	.	.	.	8	.	8
482	2	3	.	.	3	2	2	1
489	2	3	.	.	.	7	2	6
490	2	3	.	.	.	.	.	2
493	2	4	.	.	.	4	.	2
460	2	2	.	.	.	.	2	5

The expectation is closely realised, though there is occasionally some difficulty in deciding the position of a family. The relative proportions of the different grades of yellow have to be taken into account. Thus, families 443, 492 and 496 are put with type 2 on account of a strong mode at 3—comparable with other members of the group, the parental grade of which is known. Families segregating into yellow and cream showing a strong mode at 3 or 2·5 are put with type 3. Where the parent grade is known, there is of course no difficulty in assigning a family to its proper class. There are variations in the type of segregation within a group: thus families 425 and 443 show very different types of segregation, probably due to the action of further minor modifying factors. It is, however, clear that one main gene is concerned with the intensification of **P**. Further evidence is provided by a classification of the backcross itself:

		Grades 3 and 2·5	Grade 2
		PQ PQ PQ	PQ
		PQ Pq pQ	pq
Obtained	... ...	52	15
Expected	... ...	50·25	16·75

The numerical results for the segregating families are as follows:

		Yellow (**P**)	Cream (**p**)
Observed	... ...	308	104
Expected	... ...	309	103

GENERAL DISCUSSION.

The series of experiments described in this paper will illustrate admirably the fact that the sharpness of the distinction between the dominant gene **P** and the recessive **p**, depends upon the genetic background. So far the lowest grade of yellow encountered has been 0·5, and it would be almost impossible for anyone unfamiliar with the material to distinguish this grade with certainty from grade 0. By varying the genetic background the type of segregation can be correspondingly varied. By crossing a heterozygote with a 0 carrying plus modifiers, segregation can be made as clear and definite as in Pima × Grenadines White Pollen, or on the other hand, segregation can be modified to make a series of yellow down to cream which will defy analysis.

Single "point mutations" in cotton—so far, these have only been noted in Sea Island and Egyptian—comprising the types white flower, naked seed and crinkled dwarf, always segregate in a clean-cut manner when crossed with the type from which they originated. No case has
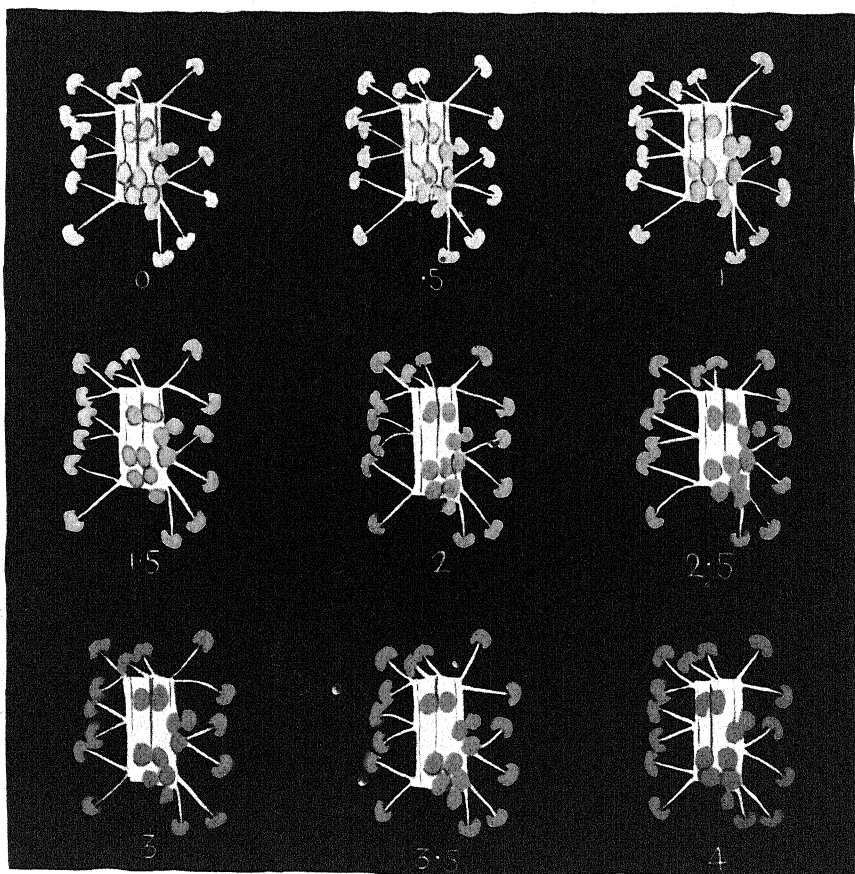
yet been encountered of segregation as clear-cut in an inter-specific cross—there is always segregation of modifiers as well. Sea Island white flower can be used for cutting out intermediate grades of yellow corolla in an interspecific cross since it has lost nothing but the main gene for yellow, **Y**, and still retains its constellation of plus modifiers. It may here be stated that species hybrids in cottons differ from varietal hybrids in the extent to which differences occur in modifying factors. In a species hybrid each main gene is apparently accompanied by a group of modifiers which have the effect of diluting the character in steps down to the recessive, and in certain cases may obscure entirely the distinction between dominant and recessive.

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EXPLANATION OF PLATE X.

Illustrating a series of 9 grades of anther colour in cotton. For full explanation see text, p. 388.

AN ATTEMPT TO CROSS HARE AND RABBIT.

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AMONG some breeders of rabbits the possibility of a Hare-Rabbit cross is credited. Richardson[1] quotes a number of instances and successful experiments have been reported by Kuiper[2] working in conjunction with Houwink, in which they state that the offspring are fertile, but a full account of the experiments has apparently not yet been published although many points of very special interest are involved, such as for instance the fertility in the succeeding generations and the inheritance of the red colour of the hare's flesh. Since the cross would form a means of testing the inheritance of dark and light coloured meats, the hare having red muscles and the rabbit mainly white ones, we attempted to make it by means of artificial insemination.

Yamane and Egashira[3] attempted to make the cross by artificial insemination of doe rabbits with the sperm of the Japanese hare (*Lepus timidus ainu*), but failed to obtain any offspring, although rabbits inseminated with rabbit sperm at the same time were fertile.

Insemination was adopted owing to the difficulty of keeping or breeding hares in captivity, and to the possibility that if female rabbits are tethered or turned out with hares in an enclosure insemination might be due to a wild rabbit interloper.

We have had considerable experience in artificially inseminating rabbits and could rely on getting good results from rabbit to rabbit. The rabbit ovulates normally only after copulation (10 hours), so that before doe rabbits were inseminated with the semen of the hare they were mated with sterile (vasectomised) bucks. The male hares were shot in the field and then as soon as possible taken to the laboratory, where the does were inseminated. The following experiments were made:

(1) 30 *March*, 1925. A hare was shot about 9 a.m. at the University Farm; the testes and epididymes were removed and minced in ·15M NaCl about 12.30 p.m. and two does were inseminated but no young were produced, although one made a pseudopregnant nest at the 21st day. Four other does were also inseminated, but these were subsequently found to

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[1] Richardson, *Fur and Feather*, 1923, April 13, 20 and 27.

[2] Kuiper, *Genetica*, VII. 471, 1925.

[3] Yamane and Egashira, *Dobutsugaku Zasshi* (Tokyo), XXXVI. no. 430, 1924.

be sterile. There was plenty of sperm present in the epididymes but it was not motile. It was thought possible that their lack of motility was due to the testes being left in the body of the animal for some time after death, for Redenz[1] has described this as a post-mortem change.

(2) 8 *October*, 1927. Two male hares were shot at Babraham at 1.30 p.m. and 3 p.m.; their testes and epididymes were removed from the body into tubes, kept in a thermos at 60° F., and examined in the laboratory at 5.15 p.m. Very little fluid was present in the lower epididymes; in one hare no sperm at all were found, while in the other there were only a few sperm but none were motile. The testes weighed 1·6 and 1·6 and 2·0 and 1·8 grm.

(3) 10 *October*, 1927. Three male hares were shot at Brandon at 3 p.m.; the testes were removed into tubes immediately after death, and one epididymis from each hare was cut off and immersed in Ringer's solution. They were examined in the laboratory at 7 p.m., but only one or two sperm could be seen and these were not motile. At the time it was thought possible that these hares were too young and were not yet of breeding age.

(4) 10 *October*, 1927. Six male hares were shot at Balsham; their testes and epididymes were removed into tubes immediately, and were examined in the laboratory about 4 hours after killing. No motile sperm were found in any of these, but in one, which was certainly an adult, there was a lot of sperm, but none were motile. Many of the sperm in the epididymes consisted of heads separated from the tails as though they had been dead for some time. The testes of this animal weighed 1·8 and 1·9 grm.

(5) 12 *October*, 1927. The testes and epididymes of an adult hare, which had been removed from the animal directly after killing, were examined in the laboratory about 3 hours after it was shot. The testes were small, and only one or two sperm were seen, which were not motile. The majority consisted of separated heads and tails.

It was concluded from the results of these last four attempts that the month of October is out of the breeding season for hares, and that after the breeding season is over the sperm do not continue to live in the epididymes (as they do in the hibernating bat (Courrier[2])) but are absorbed. It was then decided to wait until the height of the breeding season before beginning other experiments.

(6) 6 *April*, 1928. A male hare was shot at Wilbraham at 3.45 p.m.

[1] Redenz, *Würzburger Abhand. a. d. Gesamt. d. Med.* XXIV. 1926.

[2] Courrier, *C. rend. Soc. d. Biol.* LXXXVII. 1365, 1922 and LXXXVIII. 1163, 1923.

The testes and epididymes were removed into tubes immediately the animal was killed. The lower epididymes which were full of thick white fluid were minced in Ringer's solution. The fluid was swarming with sperm, of which nearly all were motile, and eight doe rabbits were inseminated after mating with a sterile buck at 5-6 p.m., but none produced young. Three of these does made pseudopregnant nests with fur at the 21st day, showing that ovulation had occurred. The upper epididymes only contained a little fluid with a moderate number of sperm, about half of which were motile. The testes weighed 10·3 and 10·5 grm.; a few sperm were obtained from them, but only a few of these were motile. The minced lower epididymes were left in Ringer's solution in a covered watch glass standing on the laboratory bench and were examined periodically, the room temperature at that time being about 55° F. At 9 a.m. on 7 April, about 60 per cent. were motile and some slightly bent; at 5.30 p.m. on 8 April, about 25 per cent. were moderately motile and most were bent; at 4 p.m. on 9 April, only about 1 per cent. were slightly motile, while at 9 a.m. on 10 April, none were motile.

The length of time these sperm lived in the Ringer's solution used would appear to be sufficient proof that they were not damaged by this solution (as a dilutant for rabbit sperm, to be injected immediately as this was, it is quite successful), and the length of time they survived would, in the case of rabbit sperm, have led us to believe that these sperm were capable of fertilisation for 2 days after they were removed from the animal.

(7) 9 *April*, 1928. A male hare was shot at Histon at 11.30 a.m. and the testes and epididymes were removed to glass tubes as soon as it was killed. The lower epididymes were minced in Ringer's solution and six does were inseminated after sterile coitus at 12.10–12.40 p.m. None of these does produced young, but two made pseudopregnant nests at the 17th and 19th days respectively. The fluid from the lower epididymes showed very large numbers of sperm, of which about 90 per cent. were motile. The small pieces of minced lower epididymes were squeezed out with forceps, and a fluid obtained which contained much tissue with but little Ringer's solution, and two more does were inseminated with this at 12.45 p.m. Neither of these produced young, but one made a pseudopregnant nest on the 18th day. The upper epididymes were also minced in Ringer's solution and the pieces squeezed out, and the fluid (which contained many sperm, 50 per cent. being motile) was inseminated into a doe at 2.30 p.m.; this doe did not become pregnant, but made a pseudopregnant nest at the 17th day. The pieces

of epididymes, after squeezing out were put back in the Ringer's solution, and stood covered on the bench, at room temperature (55° F.); about 5 per cent. were motile at 10 a.m. on 10 April; one or two were feebly motile at 9 a.m. on 11 April, and none were motile at 9 a.m. on 12 April. The testes weighed 10·3 and 12·1 grm.; a few sperm were obtained from them but none of these were motile. The sperm were very similar to those of the rabbit in appearance.

In surveying the results of these experiments the failure to obtain the cross might be attributed in experiment (1) to the method of keeping the sperm, and in experiments (2) to (5) to the attempts being made outside the breeding season of the hare. The finding of few and degenerating sperm in the epididymes which contained but little fluid (*i.e.* the secretions of the tract were not being produced), together with the comparatively small weights of the testes (the six testes weighed in October averaged 1·8 grm. each, as compared with an average of 10·8 grm. each for four obtained at the beginning of April), suggest that there is a definite breeding season in the male hare, influencing spermatogenesis and the physiological functions of the epididymis, as has been described by von Lanz[1].

The failure of experiments (6) and (7), done as they were under the optimum conditions, viz. at the height of the breeding season early in April, at a short time between death and insemination ($\frac{3}{4}$–2 hours), and at a low temperature (55° F.), as well as the evident viability of the sperm, shown by its remaining motile for 3 days outside the body in the fluid used as a dilutant, would appear to indicate that the cause was due to the incompatibility of the cross, for under similar conditions with rabbit sperm we should have expected to obtain full fertility.

This failure is of course not conclusive evidence that the cross is impossible under all circumstances, but it is sufficient to show the need for exact particulars of the circumstances under which it can be made from those who claim its possibility.

We are indebted to the following gentlemen who have supplied us with hares: Mr Lewis Kent, Mr Webster Watts, Mr R. S. Hicks, Mr Stanley Chivers and Mr K. Fair.

[1] von Lanz, *Zeit. f. d. Gesamt. Anat.* Abt. I. 80, 1926.

PRIMULA KEWENSIS AND ITS DERIVATIVES

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(Students of the John Innes Horticultural Institution.)

(With Three Plates and Nineteen Text-figures.)

CONTENTS.

INTRODUCTION.

THE genetical study of the hybrid, *Primula kewensis*, begun in 1911 by Miss F. M. Durham and myself, was discontinued soon after the publication of our results in 1916, for it had become evident that no further progress could be made without extensive cytological investigation. Some of the experimental material was, however, kept, and in 1923 Mr W. C. F. Newton was attracted to it, and began that series of observations which has reconciled the genetical with the cytological behaviour of the hybrid. The account of the work which follows was in large part written by Mr Newton, but at his request I have rearranged his manuscript, and have added an account of the breeding experiments. Except in rare instances the interpretations here given of the genetical

and cytological observations proceeded from him, or we had agreed on the conclusions reached. In the final preparation of the paper for publication I have been assisted by Mr C. D. Darlington. I wish also to acknowledge the help given in the cytological investigations by Mr H. Bruun, in the spring of 1924. [C.P.]

Primula kewensis appeared originally at Kew in 1900 among seedlings of *P. floribunda*, and was supposed to be a hybrid between that species and *P. verticillata*, a hypothesis that was immediately confirmed by the production of the plant as a result of pollination of *floribunda* by *verticillata*. Its history will be found in more detail on p. 423. On several occasions this highly sterile hybrid has produced fertile flowers, the seed of which gives rise to a fertile giant form. Within limits to be described more closely below, the fertile form breeds true.

The cytology of the parent species and of the hybrids was investigated by Digby (1912) and by Farmer and Digby (1914). They found that the diploid number of chromosomes, alike in the parent species and in the sterile hybrid, was 18, while in the fertile giant form the number was 36. This was the first example of a sterile hybrid producing seed, the change being associated with a doubling of the number of chromosomes, and in consequence *P. kewensis* is much quoted in genetical literature. Unfortunately its constitution has usually been misunderstood as a result of the suggestion by Farmer and Digby that the increase in chromosome number was due to fragmentation, and not to longitudinal splitting, of each chromosome. The difficulties arising from the adoption of this theory have been pointed out by several workers, including Winge (1917), Winkler (1920) and Renner (1924).

The problems that arise in connection with the hybrids dealt with in this paper are as follows:

(1) What is the nature of the doubling of the chromosomes, *i.e.* is their increased number due to transverse fragmentation, or is each chromosome represented twice?

(2) What is the reason for the high degree of sterility of the diploid hybrid, and at what stage in its life-history does the change in chromosome number leading to fertility take place?

(3) Why is the tetraploid fertile? Why does it breed relatively true and yet throw a comparatively small number of aberrant forms?

So far as possible these questions will be dealt with in order, but they are closely interrelated and a certain amount of cross-reference is unavoidable.

I. CYTOLOGY.

1. SOMATIC CHROMOSOMES.

In the earlier accounts of the chromosomes of *P. kewensis* (Digby, 1912; Farmer and Digby, 1914) no description was given of the somatic chromosomes of the hybrid, tetraploid or diploid, or of those of the parent species. Indeed, the only illustration showing the chromosomes at all is one purporting to represent the "fully developed chromosomes" (Digby) which are actually not even countable. Their conclusions (Farmer and Digby, 1914) with regard to the origin and construction of the tetraploid were drawn from observations of the reduction division, yet it is in somatic divisions, whether sporophytic or gametophytic, that the chromosomes are most constant in respect of size and structure. They measured for comparison, presumably from side views, 20 chromosomes "at early anaphase of the heterotype division," a stage at which chromosomes show the minimum constancy of form, and the maximum variation in size. They state that "Every care was taken to secure that only those chromosomes were selected with regard to which no doubt as to the accuracy of the measurements could be raised." At this date, in the absence of illustrations and also of the actual figures of the measurements, no one is likely to raise any doubt on this point, but in regard to an average of measurements of chromosomes, the different members of the complement in different cells, in different fixations and at a rapidly changing stage of division, we do indeed question the validity of the difference between 1.262μ and 1.022μ, and that between 1.11μ and 0.874μ. Our own observations of the pollen mother-cell divisions in the tetraploid entirely fail to support Farmer and Digby's conclusions, and observations of the somatic divisions prove that the somatic complement is homologous with two complete sets of the diploid hybrid.

The chromosome sets of *floribunda* and *verticillata* are, as far as we can tell, indistinguishable, the range of size being the same. They contain several pairs that are undoubtedly comparable: for example, one short pair in each has a median constriction which gives it a characteristic V-form; a rather longer pair in each has a sub-median constriction (Text-fig. 1, *a* and *b*).

In the diploid hybrid we find the same range of size and the same forms as in its parents (Text-fig. 1, *c*). Finally, in the tetraploid (Text-fig. 1, *d*) we find the same chromosome types, both in respect of size and of structure. The chromosomes of each of the four forms vary in length, the range of size being from 1.2 to 2.5μ. In fact, the difference

amongst the individual chromosomes of each form is at least as great as
the 100 per cent. difference between those of the diploid and the tetra-
ploid which Farmer and Digby attempted to prove.

More direct evidence on this question is derived from a plant (18/12)

Text-fig. 1. Mitoses in root-tips of
a, *floribunda*, metaphase. × 5700.
b, *verticillata*, late metaphase. × 5700.
c, *kewensis* diploid, anaphase. × 5700.
d, *kewensis* tetraploid, metaphase. × 5700.

obtained by selfing a (presumed) tetraploid, from *kewensis* tetraploid × *floribunda*. The plant 18/12 was examined by Farmer and Digby, who concluded that it had 18 chromosomes (Farmer and Digby, 1914, pp. 4–5). But in 1923 this plant was re-examined by us and found to have 26 chromosomes, indicating that its parent was a triploid. Now on the fragmentation hypothesis a triploid should have 9 undivided and 18 divided chromosomes, and a plant derived from it would be expected to have both types of chromosome represented. Actually however there is no greater range in the size of the chromosomes of 18/12 than in either the diploid or the tetraploid hybrid (Text-fig. 6, p. 422).

There is thus no valid evidence against, while there is direct evidence for, the hypothesis of longitudinal splitting, a hypothesis having the further advantage of rendering the genetical behaviour of *kewensis* 4n, which is inexplicable on the fragmentation hypothesis, comprehensible and conformable with that of other plants.

2. Meiosis in the diploid and tetraploid hybrids.

The diploid hybrid. In this plant 9 loosely paired bivalent chromosomes are formed and there is as a rule no irregularity, 9 chromosomes being segregated to each of the four spores of the tetrad. Very rarely a lagging chromosome has been seen, but we have not observed any fertile plant showing more regularity in meiotic behaviour. The pollen develops normally for a time but large numbers of small and empty pollen grains are found later on, and at the time of dehiscence of the anthers there is very little well-formed pollen.

The tetraploid hybrid. In the pioneer work of Digby (*op. cit.*) an excellent account is given of the later stages of reduction in the tetraploid, though in our opinion her figures do not afford decisive evidence for the occurrence of either parasynapsis or telosynapsis, nor do our preparations supply the deficiency. This controversial question is not however of importance as far as the present discussion is concerned. In diakinesis the number of separate bodies present is usually 17, and this is also the case in metaphase plates of the first division. Sixteen of these bodies are obviously bivalents; the seventeenth is the quadripartite ring chromosome, figured and described by Digby at a time when such bodies were not so well known as they are now. This quadrivalent occurs frequently at diakinesis and in metaphase of the first reduction division. The counts given by Digby are hard to understand, "Very rarely 18 bivalent chromosomes all of the same size are to be found; the greater number show 17 bivalent chromosomes together with one quadrivalent

chromosome, a few show 16 bivalents and one quadrivalent, and one case was found of 15 bivalents and 2 quadrivalents" (pp. 374–5). The second case providing the majority of the counts, and the fourth case, seem to point to a greater number of chromosomes than 36 in some of the plants examined by Digby. In our own observations of plants with 36 chromosomes, when only one quadrivalent occurs there are, in undisturbed plates or in complete nuclei, only 16 bivalents in addition, 17 bodies in all. Occasionally we have observed a second and even a third quadrivalent, showing that single quadrivalents may be of three kinds. In plants with 37 chromosomes there may however be an extra body which can usually be recognised as a univalent. Plants with chromosome numbers deviating from the normal are formed as the result of irregularities in meiosis. It should be emphasised that inequalities in the hetero- and homotypic divisions occur more rarely in the sterile than in the fertile form.

The occurrence of quadrivalent chromosomes is of considerable theoretical importance, and their mode of formation requires consideration. It is obvious, from the formation of 9 bivalents in the diploid hybrid, that in the tetraploid there are 9 sets of four homologous chromosomes, *i.e.* any one of the four is capable of pairing with any other one. In at least three sets, all four chromosomes may unite to form the quadrivalents, as is shown by the occasional observation of three quadrivalents in a single cell. The genetical significance of the above facts will be discussed later (p. 413).

3. Reproduction in the diploid hybrid.

In spite of many attempts we have failed to obtain seed from self-pollinated flowers, nor does the diploid hybrid set with the pollen of either of the parent species. Seed has however been obtained by pollinating *floribunda* with pollen of the hybrid. Plants raised from this seed closely resemble *floribunda* whilst showing traces of hybrid influence (Text-fig. 17). Five such plants were examined and found to have 18 chromosomes. In one the reduction division shows, besides normal divisions with 9 bivalents, cells in which there are 8 bivalents and 2 univalents. It is a point of some interest that these back-crosses should show more irregularity in meiosis than the original hybrid.

This result in some degree resembles that of Goodspeed and Clausen (1922) in back-crosses of (*Nicotiana sylvestris* × *Tabacum*) × *N. sylvestris*, from which they obtained only pure *sylvestris* plants. They explain their results as due to zygotic elimination of all combinations not genetically identical with *sylvestris*. But in our case no true *floribunda* appeared,

although chance distribution of the chromosomes would give $(\frac{1}{2})$ 9, or 1 in 512, gametes containing a complete set of *floribunda* chromosomes. In view of the very large number of shrivelled pollen grains in the hybrid, a high proportion of those surviving might be expected to contain such a chromosome complement. Our result would appear to indicate crossing-over between *floribunda* and *verticillata* chromosomes. To prove this point we should require the evidence of linkage, *i.e.* two pairs of allelomorphs showing "coupling" and "repulsion," evidence not as yet obtained. Nevertheless, as will be shown later, the general body of evidence derived from the genetical study of the tetraploid hybrid and of triploid plants favours the view that interspecific crossing-over occurs, at least between certain chromosomes. In the case under consideration the differences between the individual plants appear to be of a different order to those between 18- and 19-chromosome plants (see Text-figs. 18 and 19).

Although we cannot explain the apparent absence of gametes bearing the nuclear complement of either parent species, we suggest that the sterility of the hybrid is due to the formation at meiosis of non-viable recombinations. The complete sterility of the female side as compared with the partial sterility of the male may be a consequence of a difference in viability between male and female gametes of the same nuclear constitution, but we would suggest a simpler possibility. If the effective gametes are the same on the male and female sides, each hybrid ovary would nevertheless bear only a very small number, relatively to the number applied in pollination. Thus the chance of obtaining viable zygotes would be very greatly reduced when the hybrid is the female parent, and if a certain number of fertile seeds is required to bring about the development of a capsule, this number may be attained when pollen of the hybrid is used on *floribunda* though not in the reciprocal cross. The triploid plant 18/12 referred to above is also more sterile as female than as male, possibly for the same reason; there is no indication that the proportion of sterile spores is greater on the female side (p. 420). The study of the back-crosses of the diploid hybrid to *floribunda* is only beginning and a full account of their behaviour must be deferred to a later paper.

4. The Origin of the Tetraploid Hybrid.

The first occasion on which the diploid hybrid bore seeds was in 1905, in the nursery of Messrs Veitch. Not until 1926, when a fertile inflorescence appeared on a plant at this Institution, was it demonstrated that the

vegetative parts of a fertile stem were tetraploid[1]. The plant itself was exhibited at the meeting of the British Association in 1926 with the tetraploid and diploid portions attached to one another (Text-fig. 2). The tetraploid number of chromosomes was observed in the bracts of the inflorescence. If a doubled cell gives rise ultimately to an inflorescence, that inflorescence is fertile, and from it independent tetraploid
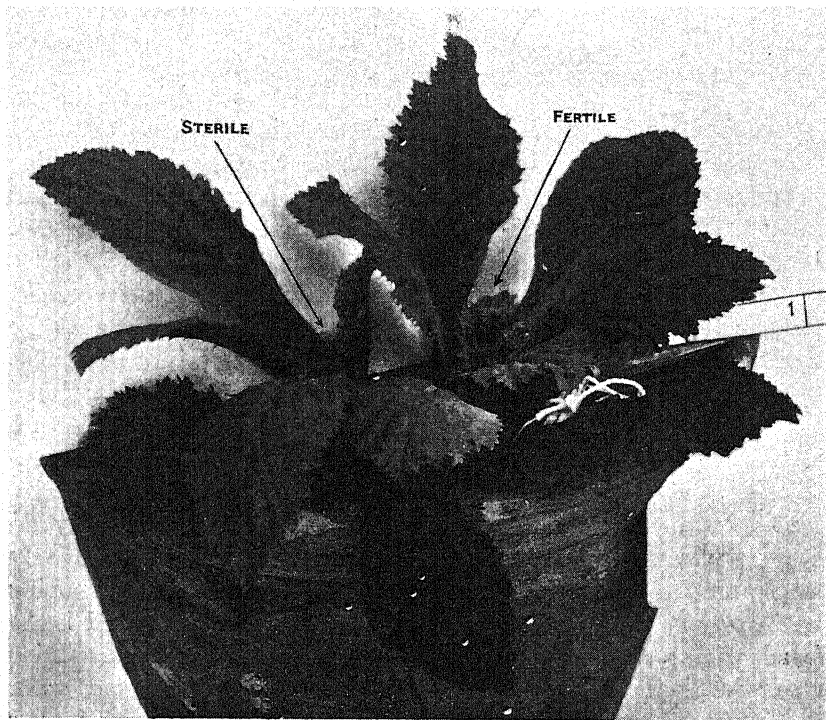
Text-fig. 2. *Kewensis* diploid, showing the crown with broad leaves from which a fertile inflorescence arose.

plants are produced. The most obvious hypothesis to account for the doubling is that a cell division is arrested after the division of the chromosomes, but before their separation into two groups. Such an arrested division would result in each chromosome being represented twice, as is demonstrably the case in doubled somatic cells observed by us in *Pisum* and in *Tulipa Batalini*. It does not appear that somatic doubling is more common in hybrids than in pure-bred plants. It is

[1] Further details of the various occasions on which the tetraploid form has arisen are given on p. 424.

possible that a reason for the greater commonness of polyploid series in plants than in animals is that, owing to their different mode of growth, such "mutated cells" are more likely to give rise ultimately to germ cells. Certainly the occurrence of two sexes is not a sufficient reason for the difference as was suggested by Muller (1925), since polyploid series in bisexual plants have been demonstrated by Blackburn and Harrison in *Salix* (1922) and by Jørgensen in *Vallisneria* (1927).

5. Reproduction in the tetraploid hybrid.

P. kewensis 4n on the whole breeds true, though it gives a small proportion of aberrant plants, of which the most conspicuous, as stated by Pellew and Durham, are the mealy plants. To explain the constancy of the hybrid, and also the occasional production of maternal hybrids, Pellew and Durham considered the possibility that reproduction was for the most part apomictic. But further cytological evidence, and also the facts connected with the formation of triploids[1], have removed this possibility, though there is a gap in the direct evidence on the question in that actual fusion of male and female nuclei has not been observed. Reduction in the megaspore is however normal, and the egg has 18 chromosomes. The early divisions of the egg and the endosperm show 36 and 54 chromosomes respectively, indicating that normal fusion has taken place. Further, aberrant plants with 35 chromosomes give on self-fertilisation offspring with 36, 35 and 34 chromosomes, which may be regarded as sufficiently direct evidence of ordinary sexual reproduction. This being so, the rare occurrence of segregation remains to be explained.

In the meiotic divisions of the diploid hybrid 9 pairs of chromosomes are formed, which we may indicate as F_1V_1, F_2V_2 and so on. The resulting gametes would contain all possible combinations of chromosomes, one from each pair. Most of these are non-viable; a few are however viable, and these, while bearing mainly *floribunda* characters, show traces of *verticillata* (see p. 424). But in the tetraploid hybrid each chromosome is represented twice, and if 18 pairs are formed in meiosis, these may either be pairs of identical chromosomes (F_1F_1, V_1V_1, etc.) or of corresponding *floribunda* and *verticillata* chromosomes (interspecific pairing) as in the diploid hybrid. In the last case the number of possible

[1] Recent experiments on crossing tetraploids with diploids have shown that the "maternal hybrids" occasionally met with (Pellew and Durham) may be attributed to the apogamous development of gametes with the unreduced number of chromosomes (see p. 454).

combinations of chromosomes is much greater than in the diploid, and the gametes formed will be exceedingly diverse, giving on fertilisation a mixed progeny. In the former case identical chromosomes separate and the gametes will each contain a complete set of *floribunda* and *verticillata* chromosomes which on fertilisation give a uniform progeny. Thus the hypothesis of pairing of identical chromosomes (intraspecific pairing) gives a satisfactory explanation of a perfectly constant tetraploid hybrid, but it is not strictly applicable to *kewensis 4n*, for it leaves unexplained the occurrence of mealy and other more or less aberrant plants.

This hypothesis was first put forward by Winge (1917) in discussing the possible origin of fertile tetraploids from hybrids. He considered that doubling of the chromosomes might result from failure to conjugate at meiosis, followed by splitting and subsequent pairing of the identical halves. The observations of Federley on hybrids in *Pygaera* (1923) had shown that diploid gametes capable of transmitting the sum of the parental characters might be formed by such a process. But in *kewensis* diploid, as we already knew from Digby's observations (1912), pairing is regular, and there appears to be no lack of affinity between homologous chromosomes. In this difference between Winge's conception and the observed behaviour of the chromosomes appears to lie the explanation of the sporadic segregation exhibited by the tetraploid hybrid. Although the degree of affinity between specifically distinct chromosomes is such as to lead to regular pairing in the diploid hybrid, yet in the tetraploid the affinity between like chromosomes generally prevails, though interspecific pairing may occasionally occur.

For the sporadic occurrence of interspecific pairing a visible basis is observed in the quadrivalent chromosomes described above (p. 409).

The influence of quadrivalents on inheritance will depend partly on their orientation on the spindle. In those cases where a definite ring tetrad is formed it is difficult to see how anything but a chance distribution of the component chromosomes is possible, though in looser associations of two bivalents it is probable that the normal distribution resulting from intraspecific pairing occurs, a probability supported by the small numbers in which aberrant plants occur. The ratio resulting from free pairing (first shown by Muller, 1914) is as follows:

Gametic series 4FV, 1FF, 1VV.
Zygotic series 18FVFV : 8FVFF : 8FVVV : 1FFFF : 1VVVV.

On the hypothesis that assortment of the chromosomes is a matter

of chance, the calculated expectation of **FVVV** and **VVVV** zygotes is 9 in 36. But this ratio cannot in fact be expected, for, as has been pointed out, in a certain number of cases no quadrivalent is formed, and in a few, two and even three are formed, from which it follows that a single one need not always be of the same type. Moreover, it appears from the study of trivalents (*Drosophila*, Metz, 1925 and *Tulipa*, Newton, 1927) that their formation is associated with pairing of the three separate chromatids for different portions of their length, and in *Drosophila*, crossing-over between all three members of a triad has been demonstrated by Bridges and Anderson (1925). If the chromatids in the quadrivalents of *kewensis* are similarly arranged, a condition is provided for interchange between chromosomes specifically distinct. The genetical behaviour of *kewensis* supports the view that interspecific pairing occurs; the facts bearing on this supposition will be discussed below, in relation to the breeding experiments (p. 444).

We have made the suggestion (1925) that variability in mealiness might be associated with the quadrivalent which occurs most frequently. This is the most conspicuous variation as yet observed which is not associated on its first appearance with a change in chromosome number, and it is tempting to attribute new forms deviating from the normal by an increase of mealiness to substitution of a *verticillata* for a *floribunda* chromosome. But our observations of the last two years, especially those made on the group of 287 plants raised from a single fertile inflorescence on the diploid hybrid—the most reliable data we have by which to estimate variation in the normal tetraploid—have shown that there is at least one other possibility to be considered. In this group of plants variability in leaf breadth was more frequent, and this is also independent of chromosome number. Among the 287 plants, fifteen (5 per cent.) were definitely short-stalked and broad-leaved, whereas only seven (2 per cent.) showed any variation in mealiness. In view of these difficulties it is evidently premature to associate a particular character with a particular quadrivalent chromosome.

### *Variation in* kewensis *tetraploid generally associated with loss or gain of a chromosome.*

Among tetraploids raised from seed of the diploid hybrid, and also among those derived from normal tetraploids, there are a few plants different from the rest. In a group of tetraploids raised from the diploid hybrid (p. 425) 7 per cent. (20 plants) were aberrant in various respects (apart from the mealy plants and those varying in leaf breadth).

The chromosomes of six were examined and the following numbers found: 2 with 35, 3 with 36, 1 with 37. In addition a plant from a tetraploid stock at Kew (1923, p. 427) had 35 chromosomes. Of aberrant plants derived from normal tetraploids by self-fertilisation, four were selected for cytological examination. Their chromosome numbers were 35 (two plants) and 37 (two plants). A fifth plant (numbered 11/25), selected for breeding as it showed a slight variation in the distribution of meal, was not examined cytologically, but among its offspring were many plants with 37 and 36, and a few with 35 chromosomes, whence it may be presumed to have had 37 chromosomes.

The number of aberrant plants, and also the kind, varies in different families. For example, two "typical" tetraploids, almost identical in appearance, from Kew, 1923, gave, respectively, 12 aberrants in 66 and 2 aberrants in 90; moreover, the most common type of aberrant in the family of 66 plants was not present in the larger family. That "typical" tetraploids raised from seed should differ genotypically is to be expected, for they have resulted from a reduction division in which interspecific pairing and segregation may have taken place.

Two distinct 35-chromosome plants, numbered 8/25 and 19/25, have been investigated and bred from on a considerable scale, and also the plant 11/25 (chromosomes probably 37 in number). The plant 19/25 (type *B*, p. 429) differed from the normal in its lighter coloured flowers and leaves (Plate XI). On self-fertilisation it produced a family containing plants of normal appearance which proved to have 36 chromosomes, plants like itself with 35 chromosomes, and small plants, in which its characters were greatly exaggerated, with 34 chromosomes (Text-figs. 8 and 9). These last, of which there were only three, were almost completely sterile on the female side and had no pollen. The reduction division in 35-chromosome plants frequently showed one laggard chromosome which might be excluded altogether from the spores produced; consequently the number of spores with 17 chromosomes would slightly exceed those with 18, but the cases in which such loss occurs seem to be rare. A ratio of 1–36 : 2–35 : 1–34 plants is thus indicated but is not in fact realised, there being a deficiency of 35-chromosome plants and a very considerable deficiency of 34-chromosome plants (p. 430). The reduction division in the 34-chromosome plants is most remarkable as showing a high degree of disorganisation. In many cells the chromosomes fail to pair, although it is possible to find, in one anther lobe, all stages between normal pairing and complete absence of pairing (Text-fig. 3, *c–f*). It appears therefore

that pairing depends not only on the individual chromosomes, but also on their interactions. A similar case has been observed by Huskins (1927) in oats. In a race of fatuoids in which the "heterozgyote," the normal
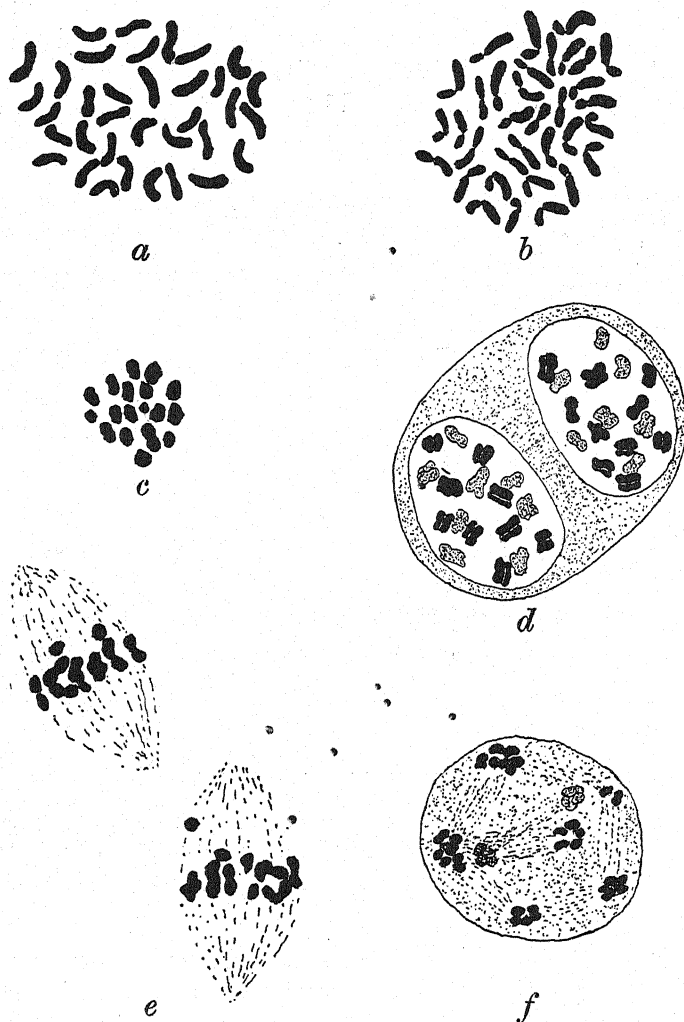
Text-fig. 3. Type B, family 51/26: (a) and (b) mitoses in root-tips at metaphase of (a) 35-chromosome plant 51^4, (b) 34-chromosome plant 51^3; (c–f) reduction divisions in 34-chromosome plant 51^1; (c) metaphase of first division, showing 2 univalents and 14 bivalents and a quadrivalent; (d) interphase, 17 bivalents or undivided daughter univalents in each nucleus; (e) side views of metaphase of the first division showing bivalents, quadrivalents and univalents; (f) anaphase of the second division, showing the irregular formation of spindles with various numbers of chromosomes. a, b, $d \times 3700$, c, e, $f \times 2250$.

and the "homozygote" have 41, 42 and 40 chromosomes, in the order
named, meiosis in the first two is regular (except for an occasional
laggard chromosome) but highly irregular in the last. Pairing at diakinesis
is comparatively rare, and the homotypic division often fails to take place.

The other 35-chromosome plant investigated scarcely differed from
the type except in having slightly smaller leaves, and a pouched corolla
tube (type C, p. 433, Text-figs. 9 and 10, and Plate XII). In its reduction
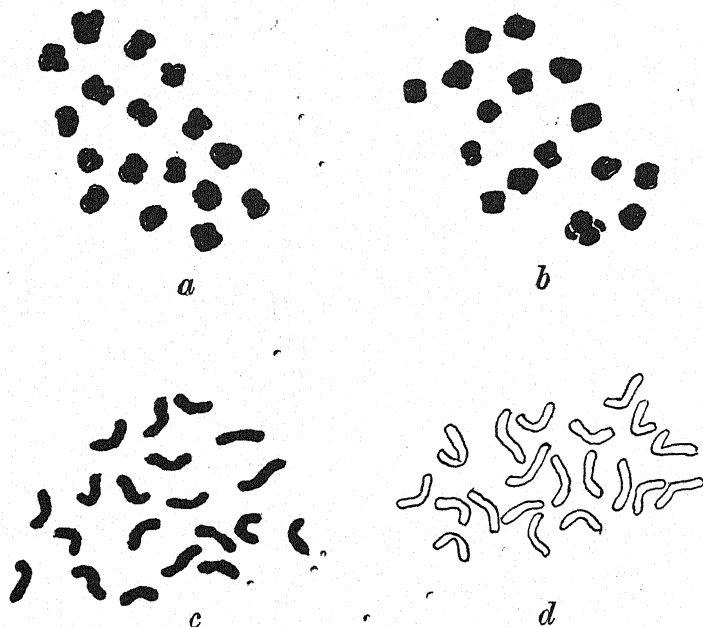
Text-fig. 4. Type C: (a–b) reduction divisions in a "spiral" dwarf plant $56^{52}/27$, with
34 chromosomes; (a) metaphase of the first division, 17 bivalents; (b) metaphase of
the first division, 15 bivalents and one quadrivalent; (c–d) mitoses in root-tips of the
"spiral" dwarf plant $48^3/26$, with 20 chromosomes. Metaphase plates. × 3700.

division the same feature is observed as in that of 19/25, viz. a frequent
laggard chromosome, which is occasionally extruded. The number of
cases in which extrusion is observed is however not enough to account
for the large proportion of 34-chromosome plants occasionally produced
by it. Among its offspring the least aberrant generally have 36 chromo-
somes, but it is difficult to distinguish between plants with 35 and
36 chromosomes, though certain peculiarities, including a tendency of
the leaves to cohere, appear to be more often associated with 35 than
with 36 chromosomes. The 34-chromosome plants also vary among
themselves, but are characterised by small size, slow growth and complete

sterility (Text-fig. 10). In the most common form each leaf is distorted as the result of its fusion along the margin with the previously formed leaf (Plate XIII). One exception to the rule that these "spiral" plants have 34 chromosomes was found, the plant in question having 20 chromosomes (Text-fig. 4, c and d). This plant was indistinguishable from its sister plants with 34 chromosomes, and shows in a most striking way how great an effect the loss of two particular chromosomes may have on the plant. Unfortunately, in common with 34-chromosome plants in general, it was of a weak constitution and died before its reduction division could be examined. Material for somatic counts had however been fixed on different occasions, and many counts were made from it before the exceptional number was regarded as established. The plant was presumably of apomictic origin.

Plant 11/25 is an example of the converse case in which an extra chromosome is present (type D, p. 438, Text-figs. 11–13, and Plate XIII). The plant was selected for breeding because it had very little meal. On selfing it gave rise to plants with 37, 36 and 35 chromosomes. The chromosomes affected are those concerned with the production of the long and homo-styled condition, for among its offspring were eleven long styled plants, none but homo-styled plants having been seen before in *kewensis*. Among the descendants of 11/25 the long style is generally associated with a peculiar type of foliage and also with a reduction in amount of meal. The chromosome numbers 35 and 37 were found in both long and homo-styled plants.

An interesting point throwing light on the origin of the chromosome aberrations has come from breeding the three plants 8/25, 19/25 and 11/25. Among their descendants, now bred to the second, and in some experiments to the third generation, a 36-chromosome type common to all three lines of descent would be expected, with a complete set of *floribunda* and *verticillata* chromosomes. But we have not been able to identify such a type. Actually the 36-chromosome plants in each line are peculiar to that line, and, if the balanced tetraploid is recovered at all, it can only be in one of them. The explanation of this fact would appear to be that the loss or gain of a chromosome follows, or is perhaps caused by, interspecific pairing and interchange between chromosomes.

6. Crosses between the Tetraploid Hybrid and *Floribunda*.

Until 1923 it was believed impossible to obtain triploid plants, the crosses between tetraploids and diploids having generally given plants

like the maternal parent (the maternal hybrids of Pellew and Durham). An exceptional plant had, however, appeared in F_2 from *kewensis* tetra-
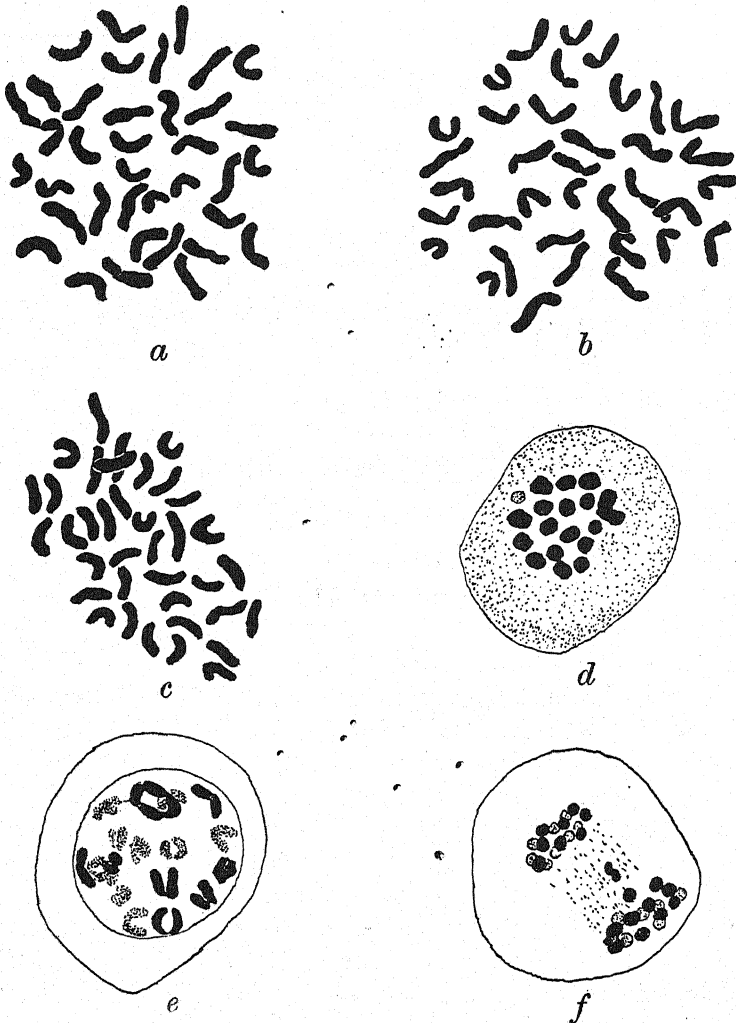

Text-fig. 5. Type D: (*a–c*) root-tip mitoses at metaphase of three 37-chromosome plants: (*a*) long styled 59/27; (*b*) and (*c*) homo-styled, 71/26 and 93/26; (*d–f*) reduction divisions in 37-chromosome plant 58^{13}; (*d*) metaphase of the first division, 16 bivalents, 1 quadrivalent and 1 univalent; (*e*) diakinesis, ring quadrivalent; (*f*) anaphase of the first division, univalent dividing on the equator. *a–c* × 3700, *d, f* × 2250.

ploid × *floribunda* (the cross having been made by Messrs Veitch) which on breeding behaved in many respects as a triploid might be expected to do. This plant (number 18/12) was examined by Digby, who concluded

that it had 18 chromosomes, and that it was of apomictic origin, the frag-
mented chromosomes of the tetraploid parent having re-united (Farmer
and Digby, 1914). In view of the striking genetical behaviour of this
plant it was difficult to believe that all that had happened was a reunion
of fragmented chromosomes, and accordingly in 1923 the reduction
division was re-examined by us. It showed the irregularity of arrange-
ment characteristic of plants derived from the meeting of gametes with
unlike chromosome numbers. At metaphase there are usually 10 biva-
lents on the equator and 6 univalents scattered in the cell (Text-fig. 6, c).
Occasionally the numbers appear to be 9 and 8, suggesting that the
pairing of the 10th bivalent is rather loose. Somatic counts showed the
chromosome number to be 26 (Text-fig. 6, a), a number which leaves
little doubt that the presumed tetraploid parent of 18/12 was in reality
a triploid. The univalents do not usually divide at the first division but
are distributed at random to one or other pole, or they are left out in
the cytoplasm. Anaphases of the second division with less than 10
chromosomes have not been observed, but their formation is demonstrated
by the chromosome numbers of plants derived from *floribunda* × 18/12.
These numbers indicate that nearly half of the pollen grains which
function have 9 chromosomes, about an equal number have 10, and
about 10 per cent. have 11 or 12. By crossing 18/12 with a tetraploid the
formation of spores with more than 12 chromosomes has been demon-
strated. This cross generally fails, or only gives a few plants which,
judged by their appearance, are tetraploids. Three have been examined
and the chromosome numbers found were 34, 35 and 36; thus the pollen
grains which proved fertile in this cross bore 16, 17 and 18 chromosomes
respectively. By neither of these crosses is it shown that pollen grains
with 13, 14 or 15 chromosomes are not formed or fail to fertilise, for
the chromosome numbers of the non-viable zygotes, of which there are
certainly a large number in both crosses, are not known. These results
may be compared with those of Kihara (1925) in his experiments on the
hybrid *Triticum Spelta* (n = 21) × *T. polonicum* (n = 14). He crossed
back the hybrid on to both parent species, and, from the chromosome
numbers of the progeny, found that the number brought in by the pollen
of the hybrid varies according to the species used as the mother. But
Watkins (1927) found no such difference in back-crossing the hybrid
Triticum vulgare (n = 21) × *T. turgidum* (n = 14).

The immense range of variation among the offspring of *floribunda*
× 18/12 (Pellew and Durham, 1916) the only cross in which 18/12 shows
a high degree of fertility, is doubtless due in large measure to the presence

of extra chromosomes, but among the 18-chromosome plants there is also a considerable range of forms which cannot be so explained.
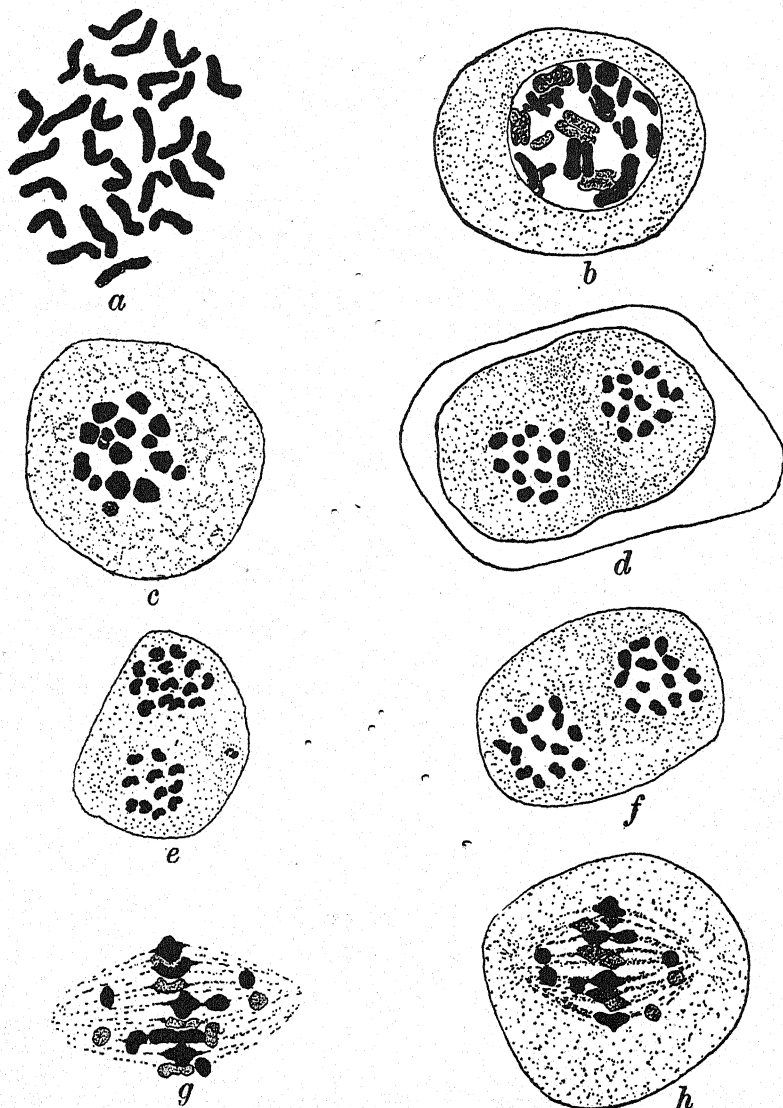
Text-fig. 6. Plant 18/12: (*a*) mitosis in root-tip, metaphase, 26 chromosomes; (*b*) diakinesis, 10 bivalents, 6 univalents; (*c*) metaphase of the first division, 10 bivalents, 6 univalents; (*d*) and (*f*) interphases, 13 + 13 chromosomes; (*e*) interphase, 14 + 11 chromosomes, one chromosome in the cytoplasm; (*g*) and (*h*) side views of metaphase of the first division showing trivalents, bivalents and univalents. × 3700.

Since it had been shown that triploid plants could exist, an attempt to obtain them was made in 1924, the tetraploid plants used being those bred for the purpose of investigating the inheritance of mealiness (p. 427). No seed was obtained. In the following year the attempt was renewed, in this case the tetraploids used being either long-styled, or heterozygous homo-styled (type D). From such crosses five plants were raised, the diploid parent in each successful cross being *floribunda* or one of its varieties. The five plants differed from the tetraploid in general habit and in the possession of a thick covering of hairs, a character derived from *floribunda*. They differed from one another scarcely at all; nevertheless on examination two were found to have about the tetraploid number of chromosomes (35 or 36), and two about the triploid number (27 or 28). The plants with the higher numbers are doubtless derived from unreduced gametes of the diploid parent. They appeared in reciprocal crosses, showing that both pollen grains and ovules with the unreduced number of chromosomes were produced.

Several cases of the simultaneous production of triploids and tetraploids, from diploid × tetraploid, have been observed recently in this Institution, *e.g.* in *Primula sinensis* (Miss D. de Winton and Mr J. Philp) in *Campanula persicifolia* (Miss A. E. Gairdner) both unpublished, in *Rubus rusticanus inermis × R. thyrsiger* (Crane and Darlington, 1928), and in *Prunus* sp. (Darlington, 1928). The triploid and tetraploid hybrids of *Nicotiana Tabacum* and *N. rustica* are also comparable (Eghis, 1927, and Rybin, 1927).

II. GENETICS.

1. THE DIPLOID HYBRID.

In view of the subsequent development of this hybrid, it is of interest to recall what is known of its production. A single plant first appeared among seedlings of *floribunda* at Kew in 1899. To confirm its parentage Mr Coutts made crosses between *verticillata* and *floribunda*, and obtained hybrids[1]. Later both Mr Coutts and ourselves (Pellew and Durham, 1916) attempted to make the hybrid again, but without success. Though viable seed was occasionally produced, it gave rise either to plants like the mother or (on two occasions) to single plants of the tetraploid *kewensis*. There is no other record of the diploid hybrid having been made, and it is believed that the only plants now surviving are from the cross made

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[1] This account is taken from notes made by Mr Bateson at Kew in 1911. It appears to have been communicated to him by Mr Coutts.

by Mr Coutts. No record was kept by Mr Coutts of the plants used in making the cross, but since long-styled plants have appeared among the descendants of the hybrid, and also in F_2 from *floribunda* × *kewensis* 2*n* we may conclude that the *floribunda* mother was long-styled.

The hybrid has never set seed in the diploid state, though it has repeatedly been self-fertilised and crossed with both parent species. Its pollen used on *verticillata* fails to give seed, though the capsules swell to a considerable size; on *floribunda* however viable seed is produced. Six flowers of *floribunda* were pollinated in 1926, and each gave rise to a capsule containing a few seeds. From these 36 plants were raised (number 14/27), an average of 6 per capsule. As *floribunda* may set about 400 seeds in a capsule, this means, supposing all the ovules are fertilised, that approximately $1\frac{1}{2}$ per cent. are capable of developing into plants.

The plants were fairly uniform, resembling *floribunda* in general character, *e.g.* size, habit, hairiness, but none were exactly like that species. They varied slightly in length and thickness of hairs, two or three having traces of meal on the inflorescence; some had leaves longer and more pointed (*i.e.* nearer the shape of *verticillata*), others shorter and broader than *floribunda*; in colour they ranged from full yellow to pale yellow. About half the plants were in some degree sterile on the male side, pollen being formed at the beginning of the season, but little or none at the end. Nearly all were capable of setting seed when self-fertilised, the exceptions being the plants with traces of meal on the inflorescence. As has been stated (p. 410) 5 plants were examined and found to have 18 chromosomes. In Text-fig. 17, a leaf of one of the narrowest leaved plants in the group is shown[1].

2. THE TETRAPLOID HYBRID.

(a) Details of origin.

The diploid hybrid has been observed to set seed on three occasions, and each time the seed produced has given rise to tetraploid plants. The first was in 1905, five years after the first appearance of the hybrid, in the nurseries of Messrs Veitch; the second, in 1923, at Kew; and the third, in 1926, at this Institution. In the early records fertility was supposed to be associated with a change in the position of the stigma resulting in an inflorescence with long-styled flowers, the rest of the plant

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[1] Seed raised from the fertile plants of this group in the past season gave a mixture of homo- and long-styled plants in every case. Thus the hybrid gametes fertile on *floribunda* are those bearing the long-styled allelomorph which must have been introduced originally by *floribunda*, for *verticillata* is known only in the homo-styled condition.

being short-styled (*Gard. Chron.* 1911, p. 378). But this observation was probably inaccurate, for in conversation with the foreman who made it we found that he had not understood the real distinction between long- and homo-styled flowers. Of the second occasion at Kew nothing is known beyond the fact that ripe seed was found on the diploid hybrid, from which fifty-five plants were raised. These plants were seen by us; they appeared to be normal tetraploids, and no striking variation was observed among them, but of three plants given to us by the Curator one was found afterwards to have only 35 chromosomes (plant no. 8/25, p. 433).

The change to fertility at this Institution was first noticed at the flowering stage. The three flowers forming the first whorl of the flower stem were slightly larger than normal, with large anthers protruding at the apex of the corolla tubes. Their pollen was examined and found to contain many more good grains than is usual in the diploid hybrid, and the stigmas were pollinated with it, though self-pollination also must have occurred naturally since the stigmas were in contact with the anthers. Buds of succeeding whorls of flowers were present but failed to develop, shrivelling up as the seed of the fertile flowers ripened. The leaves of the crown from which the inflorescence sprang were found to be slightly broader than normal. Somatic cell-divisions in the bracts of the inflorescence were examined and showed the tetraploid number of chromosomes.

The three capsules on this spike contained respectively 30, 90 and 180 seeds that appeared good. The seed was sown under the number 11/27, and from it were raised 287 plants, of which 261 were fairly uniform, not differing in any marked way from the familiar tetraploid type, though showing somewhat evasive differences among themselves, and 26 were picked out as differing distinctly from the rest. The whole group of plants were roughly classified as follows:

6 plants, meal on young leaves and flower stems (see p. 427)	1 with 36 chromosomes
12 plants, leaves flat or basal leaves flat (see p. 435)	{2 with 35 ,, {2 with 36 ,,
1 plant, flowers pale yellow, leaf veins superficial (see p. 429)	—
4 plants, short broad leaves, very rugose	1 with 37 chromosomes
1 plant, long leaves, rugose	—
1 plant, very large spreading leaves	with 36 chromosomes
1 plant, dark green leaves, growth slightly restricted	—
48 plants, leaves with veins depressed, basal leaves flat	
84 plants, leaves with veins depressed, leaves upright or spreading	Leaves long and narrow in various degrees
10 plants, leaves with veins depressed, spreading habit, large plants	
104 plants with veins prominent, large plants	
15 plants, habit various	Leaves short, broad

The number of different classes in the above record is probably of little significance, for with the exception of the 26 plants which showed obvious points of difference they could not be sorted into groups without difficulty, and though each group was fairly homogeneous in general appearance, there was no sharp line of demarcation between them. Besides the variability recorded in the table there were differences in size, length of internode, erectness of flower stem and shape of corolla tube (pouched at the anthers or tapering, see. This last character is
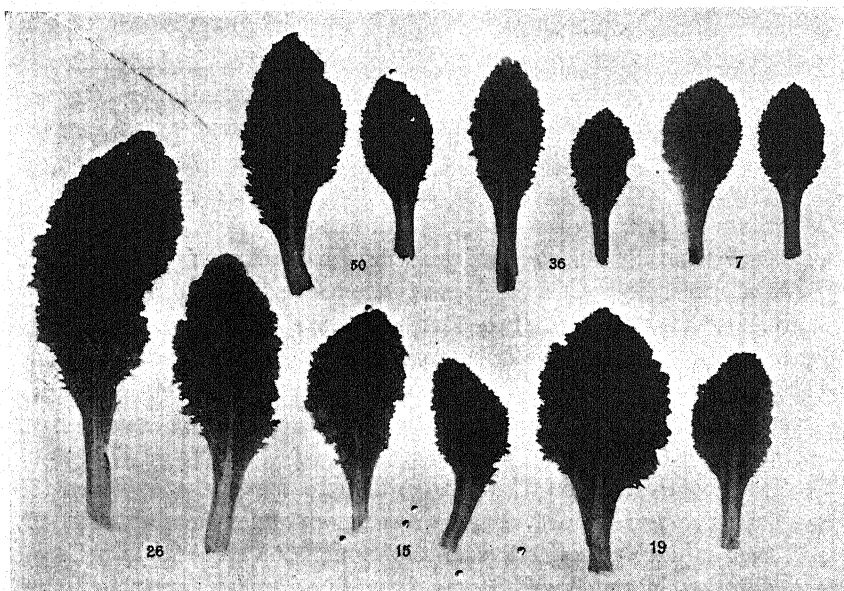
Text-fig. 7. Variation in leaf shape of tetraploid plants raised from seed of the diploid (seed no. 11/27). Young and full-grown leaves from six plants.

rarely constant in the individual, though it seems to be connected with a certain chromosome complex.

Among the 26 aberrant plants, those in the first three groups appear to correspond with the types dealt with in the following sections. None of the plants in 11/27 has as yet been bred.

(b) Mealiness.

Early in the history of *kewensis* 4n it was found that though the plant bred approximately true yet it sometimes threw plants more mealy than itself. Such plants were examined by Digby (1912) and by us, and have always been found to have the normal number of chromosomes. Breeding experiments (Pellew and Durham, 1916) showed that on self-fertilisation these plants give a number of intergrading forms ranging from the normal tetraploid condition (meal restricted to the calyx and corolla) to the fully mealy condition of *verticillata* in which only the inner surface of the corolla is without meal. The extracted fully mealy plants bred fairly true but occasionally gave plants with slightly less meal. In 1923 we decided to start experiments on the genetics of mealiness in the new stock of tetraploid plants raised from the diploid at Kew. But among the plants raised for this purpose appeared a number of variations associated with a change in the chromosome number, and to investigate these the experiments on mealiness were given up. Thus the account which follows rests mainly on the work of one season, supported by some small families grown in the years 1912–16.

Three normal tetraploids were given us by the Curator of Kew, of which one (8/25) differed slightly from the other two and was subsequently found to have only 35 chromosomes (see p. 433). The other two plants, nos. 3/24 and 4/24, were almost identical, but in 3/24 the leaves were more "ribbed," *i.e.* the veins more depressed. A fully mealy plant, raised from seed of a mealy strain grown at the Botanic Gardens, Glasgow, was also introduced into the experiment (no. 11/24). These plants were self-fertilised and the two normals crossed with the mealy plant. The resulting families are set out below. The reciprocal crosses 3 × 11 and 11 × 3 gave similar offspring and are given together:

Description	3/24 self-fertilised	4/24 self-fertilised	11/24 self-fertilised	3 × 11 / 24 and recip.	4 × 11 / 24
(1) Whole plant mealy	—	—	78	6	3
(2) Leaves slightly mealy, rest of plant fully mealy	—	—	—	30	14
(3) Leaves not mealy, rest of plant mealy	11	14	—	138	48
(4) Leaves not mealy, the bracts and outer surface of the sepals slightly mealy	10	34	—	—	—
(5) Meal restricted to inner surface of calyx and outer surface of corolla tube	44	42	—	—	—
Total number	65	90	78	174	65

Included in the above counts were the following exceptional plants:

Ex 3/24 self-fertilised

11 plants, flowers pale yellow, of which one, no. 19/25, had 35 chromosomes (p. 429).
1 plant, leaves dark green, no. 24/25, had 37 chromosomes.
1 plant, meal restricted to base of calyx.
1 plant, corolla deeply divided.

Ex 4/24 self-fertilised

1 plant, stem hairy, no. 32/25, had 37 chromosomes.
1 plant, meal restricted to base of calyx, no. 11/25 (p. 438).
A few plants with pouched corolla tubes.

Ex 11/24 self-fertilised.

1 plant, leaves broad and flat, mealy except at base of calyx.

$$\text{Ex} \frac{3 \times 11}{24}$$

6 plants flowers pale yellow, of which one = 18/25 (p. 433).
2 plants leaves exceptionally rugose.

$$\text{Ex} \frac{4 \times 11}{24}$$

1 plant leaves dark green.

The division of the numerous grades of mealiness into five classes is somewhat arbitrary, there being a considerable amount of intergrading both in quantity and distribution of meal. Some of the intergrades are however probably not genetic, but depend on slight differences in age and vigour, and also temperature. For instance in *verticillata* there is no meal until the 6th or 7th leaf is formed. As the season advances the amount of meal increases until the maximum is reached after the plants have finished flowering in May–June. In all forms there is a corresponding increase in the amount secreted, and a source of error in the records is that for lack of space the plants could not all be kept after they had begun to flower. Had it been possible to do so, there is no doubt that a certain number in classes 2–5 could have been put subsequently into a more mealy class. In all the families included in the above table a certain number of exceptional plants appeared. It is seen that the two plants 3/24 and 4/24 were not giving the same kind of exceptions, either from self-fertilisation or from crosses with 11/24. In 239 plants raised from 3/24 self-fertilised and crossed, 17 (7 per cent.) had pale yellow flowers, while in 155 plants similarly derived from 4/24, none had pale yellow flowers, though other exceptional plants occurred. There is also a difference between the offspring of 3/24 and 4/24 in mealiness, 3/24 giving a lower proportion of mealy grades than 4/24, both on self-fertilisation and on crossing with 11/24.

In spite of the experimental difficulties the records give a certain amount of information. The fully mealy plant 11/24 (class 1) bred true

in that it gave no plants that could be included with confidence in a higher class. The normal plants 3/24 and 4/24, *i.e.* class 5, gave classes 4 and 5, and probably some (11 and 14 respectively) of class 3. Crosses between classes 1 and 5 gave classes 1–3, the greater number being of class 3, and only a few of class 1. This last result is important as it appears to dispose of the possibility that variability in mealiness is due to substitution of *verticillata* for *floribunda* chromosomes in a homologous set. On such a hypothesis the appearance of a few plants of class 1 from classes 1 × 5, indicates that the class 5 parent is producing a few **VV** gametes; consequently the corresponding **FF** gametes are expected, giving rise to a number of plants of class 5 equivalent to that of class 1. Actually however only classes 1–3 are found. Thus the experimental evidence is that the different grades of mealiness are genetical, and that they cannot be explained simply by the substitution of *verticillata* for *floribunda* chromosomes in a homologous set.

## (c) *Aberrant chromosome numbers.*

In the experiments to be described in this section, we grew a considerable number of the offspring of plants deviating from the normal, with aberrant numbers of chromosomes. In these cases a second generation was investigated on a large scale. We found that all the aberrant plants bred from were giving a very mixed progeny, and that to avoid confusion the records must be limited to the most prominent and characteristic features in each family. In this account we have further simplified the data by omitting all records of variation common to the different types investigated, *e.g.,* variation in length and breadth of leaves, and in mealiness. Our immediate purpose was to observe the characters associated with the particular chromosomes in which variation had been initiated by loss or gain of a member of the homologous set.

Type B (Text-figs. 8, 9, 12 and Plate XI).

This was the first type observed with an aberrant number of chromosomes (denoted by the letter *B*, the normal type being *A*). It is characterised by the pale green foliage with prominent veins. The leaves are slightly buckled at the margin, the bracts are folded downwards, giving the plant an unhealthy look. Often associated with this type of foliage are the following characters: pale yellow flowers, long corolla tubes, ribbed capsules, rapid growth and quick maturity combined with great freedom in flowering.

Family 51/26, *ex* 19/25: 35 *chromosomes* (see p. 428).

Description of parent. Flower stems weak. Flowers and foliage paler than normal. Capsules ribbed. Veins prominent.
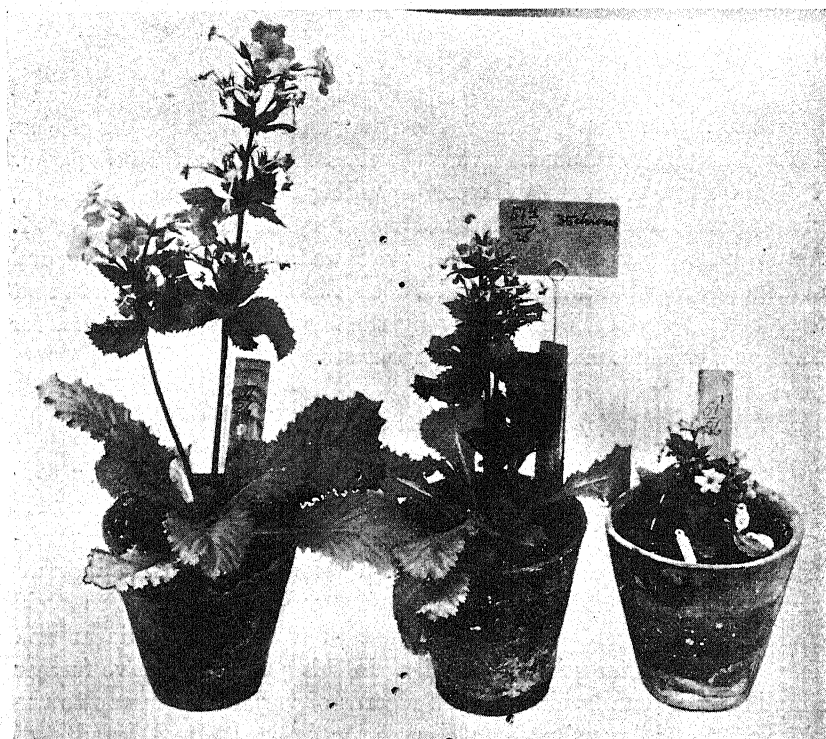
Text-fig. 8. Type *B*. 36-, 35- and 34-chromosome forms. The 36-chromosome plant (on the left) has the depressed veins of a normal plant.

Fifty-four seeds from one self-fertilised capsule were sown under the number 51/26, and thirty-one seeds germinated, giving as follows:

16 plants veins depressed, as in the normal tetraploid; 2 counted, 36 chromosomes.

10 plants type *B*; 3 counted, 35 chromosomes (2 self-fertilised, $5^p/26$ and $9^p/26$, gave families 44 and 46/27).

1 plant ? type *B*.

1 plant flat rosette, ? type *B*; counted ($84^p/26$) 35 chromosomes (self-fertilised gave family 50/27).

3 plants dwarf, leaves buckled; all counted, 34 chromosomes ($1 \times 9^p/26$ gave family 47/27).

The three main classes, viz. plants with normal venation, *B* type and 34-chromosome plants were clearly defined. The last were small, pale green, with certain features characteristic of the *B* plants but

exaggerated, *e.g.* the leaf veins more prominent, the foliage more buckled. Only one bore flowers; they were pale yellow, with the corolla tube broader than normal and the style split, frequently down its whole length, into one or more parts. No pollen was produced. Several flowers were fertilised with pollen from sister plants, and one set seed (see below, family 47/27). The capsule was elongated and pointed.

Text-fig. 9. Two plants of type *B* (19/25 and 33/27) and two of type *C* (81/26 and 37/26). All with 35 chromosomes. In type *B* the veins are less depressed and the margin of the leaf slightly buckled.

Of the two plants recorded as "? *B*," one, no. 84^p/26, with 35 chromosomes, had the flat rosette often associated with infection by a fungus (see p. 435). A small family raised from it (no. 50/27) showed that it probably belonged to the *B* class, but many of its offspring were also affected by the disease. The other ambiguous plant was not bred.

Two *B* plants were selected for further breeding, viz. no. 5^p/26, chromosomes not counted, gave family 44/27, and no. 9^p/26, 35 chromosomes, gave family 46/27.

Family 44/27.

Ex 5/26. Three flowers self-fertilised. Seed grading from very small to good. Gave 97 plants as follows:

42 plants normal venation; flowers pale yellow.
48 plants about type *B*; 1 counted, 35 chromosomes.
1 plant robust, ? extra chromosome.
5 plants flat rosettes; 1 counted, 35 chromosomes.
1 plant, flowers as in 34-chromosome plant; foliage peculiar, 35 chromosomes.

Family 46/27.

Ex 9/26. Thirty-five chromosomes. Two flowers self-fertilised. Seed grading from very small to good. Gave 116 plants as follows:

29 plants, normal venation, flowers pale yellow.
77 plants about type *B*.
4 plants frilled leaf margins, ? extra chromosome.
3 plants new type of leaf, very long.
3 plants flat rosettes.

The two families (44/27 and 46/27) are alike and also resemble family 51/26 in containing plants with normal venation or with *B* foliage. They differ from each other in the relative numbers of the two forms, and from 51/26 in the complete absence of the 34 chromosome types, a difference not remarkable in view of the small number obtained in 51/26. More remarkable is the fact that the plants with normal venation are not strictly normal either in foliage or flower colour, but are distinctly paler in both. The pollen of several "normal" and *B* plants was examined; in the former it was well-formed, in the latter approximately 50 per cent. appeared good.

There remain three families to record, bred from plants in 51/26:

Family 47/27.

Ex 34 chromosome plant in 51/26 × 9/26 (35 chromosomes) see family 46/27 above.

One flower set, giving 12 good and a few doubtful seeds, of which 5 germinated. The five plants were all type *B*.

Family 48/27.

Ex 4/24 (unrelated tetraploid) × 5/26 (see family 44/27 above).

Much seed was produced and germinated, only 24 plants were grown. Of these, three were type *B*, the rest about normal.

Family 50/27.

Ex flat rosette in 51/26, 35 chromosomes, self-fertilised.

Many seeds germinated. 28 plants grown, as follows:

> 3 plants normal venation.
> 1 plant type *B*.
> 5 plants about type *B*.
> 19 plants flat rosettes (some near type *B*).

The next family to be described came from a plant, 18/25, which resembled 19/25 in many respects. Its chromosomes were not counted.

Family 50/26, ex 18/25 (see p. 428).

Description of parent. Flowers paler than normal. Capsules slightly ribbed. Veins prominent. Bracts slightly mealy. Leaves short and broad.

Two flowers self-fertilised produced about 140 seeds, grading from very small to good, from which 50 plants were raised under the number 50/26. Of these, 40 were grown to maturity. Among these plants with normal and *B* foliage were clearly distinguishable and in approximately equal numbers, but there were also intergrading forms not easily classified. The flower colour of plants with normal venation was generally darker than that of plants with *B* foliage but in both classes (as far as they could be separated) there was some variation. All grades of mealiness were obtained, the result expected from a plant with mealy bracts. Fully mealy plants occurred both among normal and type *B* plants.

To sum up: among the offspring of plants of type *B* (35 chromosomes) three main classes are found: (1) the original type *B*, (2) plants with foliage normal in venation but of a pale green, and flowers of a pale yellow, slightly darker than in type *B*; (3) (rare) small sterile plants with the characters of type *B* exaggerated. Cytological examinations indicate that (1) has 35, (2) 36, and (3) 34 chromosomes.

Type C (Text-figs. 9, 10 and Plate XII).

The first plant of this type found was one of three tetraploids from Kew, no. 8/25 (p. 427). It scarcely differed from the other two in general habit, but the following differences were noted:

(1) Leaves ribbed, the veins more depressed than in 3/24 (which differed in the same way as compared with 4/24. The leaves of 4/24 were of the type figured in Plate XXVIII, fig. 9, Pellew and Durham, 1916).

(2) The corolla tube enlarged forming a pouched anther sac, instead of expanding gradually at the anther level. Not all the flowers of the plant are of this type (Plate XII)[1].

(3) Very little meal in calyx and on corolla tube.

[1] The same character occurs frequently in type *D*.

This plant was not bred from until 1925 when from self-fertilisation it produced a family containing plants with 34, 35, 36 and one with 37 chromosomes. The chromosomes of 8/25 were then examined and found to number 35.
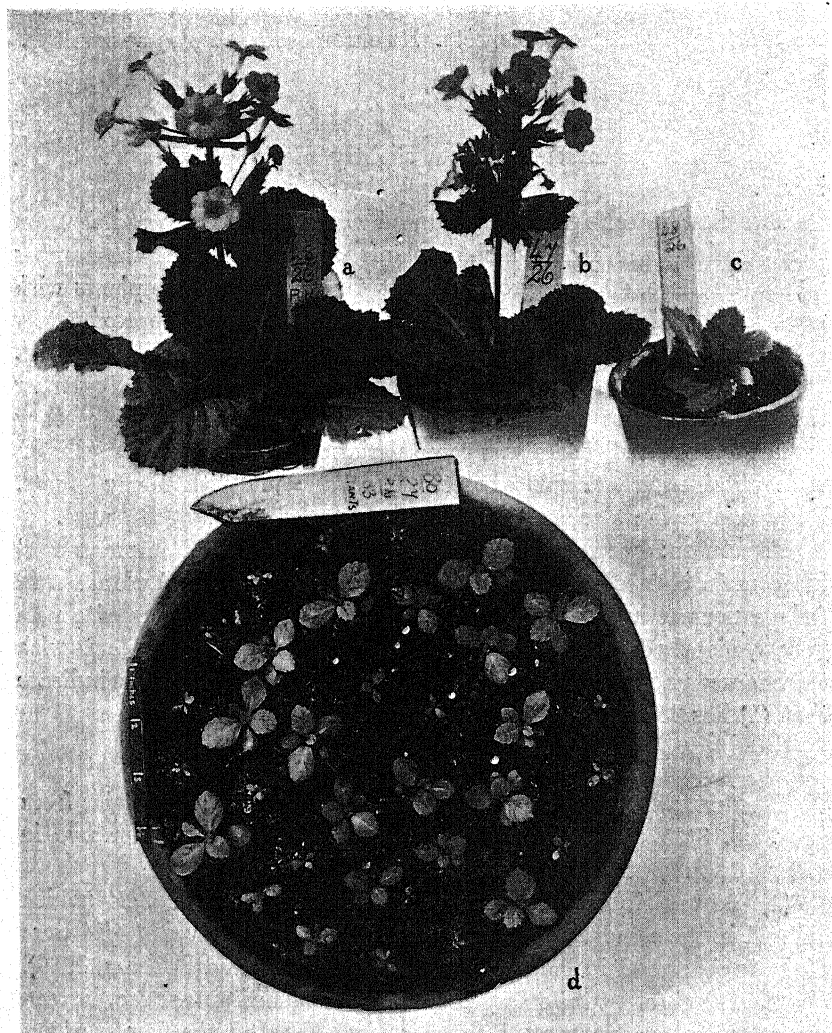
Text-fig. 10. Type *C*: (*a*) 36-; (*b*) 35-; and (*c*) 34-chromosome forms in family 47–48/26; (*d*) seedlings from a self-fertilised 35-chromosome plant (90/26) showing a mixture of small (34-chromosome) plants, and large (35- and 36-chromosome) plants. In this type 35- and 36-chromosome plants are not always distinguishable.

The various forms derived from 8/25 are as follows:

Fertile plants.

(1) Plants resembling their parent or not differing from it markedly. They may have 35 or 36 chromosomes.

(2) (*a*) The whole plant smaller than in (1).

(*b*) Leaves occasionally cohering at the base of the petiole or for some distance along the margin of the blade. Basal leaves more or less flat, instead of erect and spreading. In some cases all the leaves are flat and remain so throughout the life of the plant, but such extreme forms are probably pathological[1], for if they are repotted or stimulated to fresh growth the young leaves become erect.

The greater number of these plants have 35, a few have 36 chromosomes.

Plants with 37 chromosomes are rare. No type peculiar to the family has been identified.

Sterile plants. All are smaller than the fertile class.

(*a*) Leaves distorted by the coherence of each leaf with the next in the series, along the margin of the petiole and blade (Plate XII, figs. 6 and 7)[2], corolla tube long, funnel-shaped (Plate XII, fig. 11), sepals large, almost foliaceous. The leaves may be erect or flat.

(*b*) Very small plants, peculiar (Plate XII, fig. 8).

(*c*) Flat rosettes, leaves not fused, possibly the flat form of (*b*).

The chromosomes of eight sterile plants (of various forms) were examined, all except one had 34 chromosomes. The exceptional plant had 20 chromosomes and was presumably of apomictic origin (see Text-fig. 4, *c–d*).

From the above account it will be seen that though among the sterile and the fertile plants, different types can be distinguished, yet they are not sharply differentiated, but either form intergrading series (*e.g.* in size and in degree of flatness of the leaves) or the distinguishing character is sporadic in its expression (*e.g.* coherence of the leaves, and pouched anther sac). Hence it has only been possible to make accurate records of two classes, viz. the fertile and sterile. But even these records may fail to

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[1] Plants with perfectly flat rosettes often show signs of disease, the leaves being brittle and the base of the petiole discoloured. Miss Cayley has kindly examined such plants and found traces of the common greenhouse mould, *Botrytis* sp. Apparently the flat-leaved forms are more susceptible to the growth of the fungus than are the erect-leaved forms.

[2] The spirally twisted rosettes of these plants resemble the variety of *Plantago major* studied by Ikeno (1923).

show the gametic ratio for separate sowings of seed of the same origin may give different ratios. This is seen in three sowings of seed from 8/25 self-fertilised, which gave respectively 6, 15 and 35 per cent. of sterile plants. In two, approximate counts of the seeds which appeared good had been made. Accurate counts were not possible as the seed of all plants with abnormal numbers of chromosomes is very irregular in size. From one of the sowings (56/27) 65 per cent. of the estimated number of seeds germinated, and gave 35 per cent. of steriles: from the other (47 and 48/26), 35 per cent. germinated and gave 6 per cent. of steriles; thus a reduced rate of germination corresponded with a low proportion of steriles, and it appears that under certain conditions seeds which would develop into sterile plants fail to germinate.

The experiments relating to 8/25 are shown in Table I. The numbers of fertile and sterile plants are here given, the percentage of sterile plants being shown in the last column.

The breeding work summarised in Table I shows that all the twelve plants derived from 8/25 gave the same kind of offspring as was given by itself, whether their chromosome number was 35, 36 or 37. Although three plants (85, 88 and 77/26) failed to give any steriles, yet as they gave the various fertile types given by 8/25, and as the germination of steriles is known to be irregular, it is most improbable that they differed qualitatively from their sister plants. Of the two plants bred from with 36 chromosomes, one (81/26) had been selected as the most typical tetraploid plant in the family, and the other (76/26) was an aberrant form with a flat rosette. They both gave families containing the same types as were found among the progeny of sister plants with 35 chromosomes[1]. The chromosomes of only one of the sterile plants in these families was counted, it had 34; in addition, two fertile plants were found to have 35 chromosomes. Thus the chromosome numbers of the different phenotypes are the same, whether they come from 35 or 36 chromosome parents.

The results shown in Table I could be explained if in 8/25 a single chromosome (presumably the pair of the missing chromosome) had been modified by interspecific pairing in such a way as to bring about the frequent extrusion of one member of the homologous set in meiosis.

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[1] Among the progeny of 81/26 was the chimaera, 76/27, figured in Plate XII. The first leaves of this plant were variegated but otherwise normal. Later it was observed in the condition figured, as a sectorial chimaera of entire, non-mealy foliage, and of deeply cut, mealy foliage. The deeply cut foliage is unique in this section of Primulaceae. An inflorescence was formed on the cut-leaved sector, but the flowers failed to open and later the plant became unhealthy and was ost.

TABLE I.

Plants of type C.

I.   8/25 (35 chromosomes) self-fertilised.
II–VII. Families from plants in I.
II.   Plants with 35 chromosomes, self-fertilised.
III. Plants with 35 chromosomes, inter-crossed.
IV.   Plants with 36 chromosomes, self-fertilised.
V.   Crosses between plants with 35 and 36 chromosomes.
VI.   Plant with 37 chromosomes self-fertilised.
VII. Plant with chromosomes not counted, probably 35, self-fertilised.

(The types here tabulated are figured in Plate XII.)

	Parent plant	Seed number	No. of capsules	No. of seeds (approx.)	Progeny Fertile	Sterile	Chromosome numbers†	% of steriles
	8/25	47/ & 48/26*	2	240	79	—	1 with 37, 4 with 36, 10 with 35, 2 with 34, 1 with 20	
						5*		6*
	8/25	56/27	2	250	106‡	—	1 with 35, 5 with 34	
						56		35
	8/25	160/26	1	—	66	12		15
II	35/26	61/27	2	—	117	18	1 with 34	13
	36/26	67/27	3	—	52§	6		10
	37/26	63/27	1	50	20	12		37
	38/26	64/ & 65/27	7 (2 with many seeds)	—	164	19		10
	79/26	154/27	3	—	51‖	7		12
	85/26	78/27	2	—	34	1?		
	88/26	79/27	Several	—	43	—		
	90/26	80/27	Several	—	143	61		30
III	8/25 × 36/26	57/27	2	—	108¶	21		15
	35/26 × 8/25	60/27	2	—	26	3		10
	38/ ×37/26	66/27	1	—	47	8		14
	38/ ×35/26	68/27	1	—	34	23		40
IV	76/26	69/27	2 (1 with few seeds)	—	71	10+6?	2 with 35	— / ? 18
	76/26	70/27	3	—	126**	143	1 with 34	53
	81/26	76/27	4	—	81	15		16
V	8/25 × 76/26	72/27	1	—	81	3		4
	76/26 × 8/25	73/27	1	—	65	20		26
VI	77/26	75/27	4 (1 with very few seeds)	—	85	—		
VII	82/26	77/27	Several	—	144††	50		26

* The five steriles all came from one capsule of seed. The capsules were sown separately, but the plants raised from them were recorded together, as they were alike except in respect of the five plants.
† The numbers of plants in this column do not represent actual ratios, since special types were selected for examination of the chromosomes, irrespective of their numbers.
‡ Including 12 "fertile" discarded as young plants before flowering.
§ Including 21 "fertile" and 6 "sterile" discarded as seedlings.
‖ Including 20 "fertile" discarded as seedlings.
¶ Including 101 "fertile" and 12 "sterile" discarded as seedlings.
** Including 110 "fertile" and 45 "sterile" discarded as seedlings.
†† Including 83 "fertile" discarded as seedlings.

We do not suggest that variability in chromosome number is the only peculiarity of this type, for the number of forms in the different families points to a considerable amount of difference in genetical constitution apart from chromosome number. But we incline to ascribe both the variability in chromosome number, and the genetical phenomena in general, to changes in one chromosome of 8/25 resulting from an inter-specific pairing.

### Type D (Text-figs. 11, 12, 13 and Plate XIII).

In this type the aberrant chromosomes are those concerned with the production of heterostylism. In the parent plant, 11/25, the normal
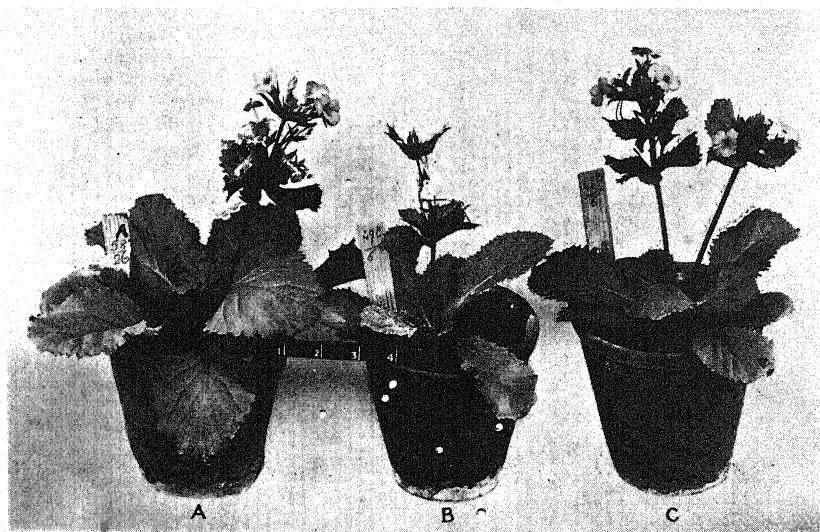
Text-fig. 11. Type *D*: *A*, homo-styled plant; *B*, mealy plant (49/26) in family 58/26; *C*, long-styled plant. In *B* the leaves approach those of *verticillata* in shape.

layer of meal inside the calyx and on the corolla tube was restricted to the base of the calyx. No other peculiarity was observed and the plant was discarded after its seed from self-fertilisation had been harvested; its chromosomes were not examined. Its offspring consisted of 27 homo-styled and 11 long-styled plants; the following chromsome numbers were found: 6 plants with 37, 9 plants with 36, and 2 plants with 35. The large number with 37 chromosomes suggests that the parent also had this number.

A peculiar habit, in its extreme form generally confined to the long-styled plants, appeared in this family (58/26). It is characterised by slow growth, thick stems, short corolla tubes, dark foliage and reduced mealiness (Plate XIII, fig. 14, Text-fig. 11, c). Not infrequently the inflorescence is slightly fasciated; sepalody of the petals, foliaceous calyces and other irregularities are common in certain individuals
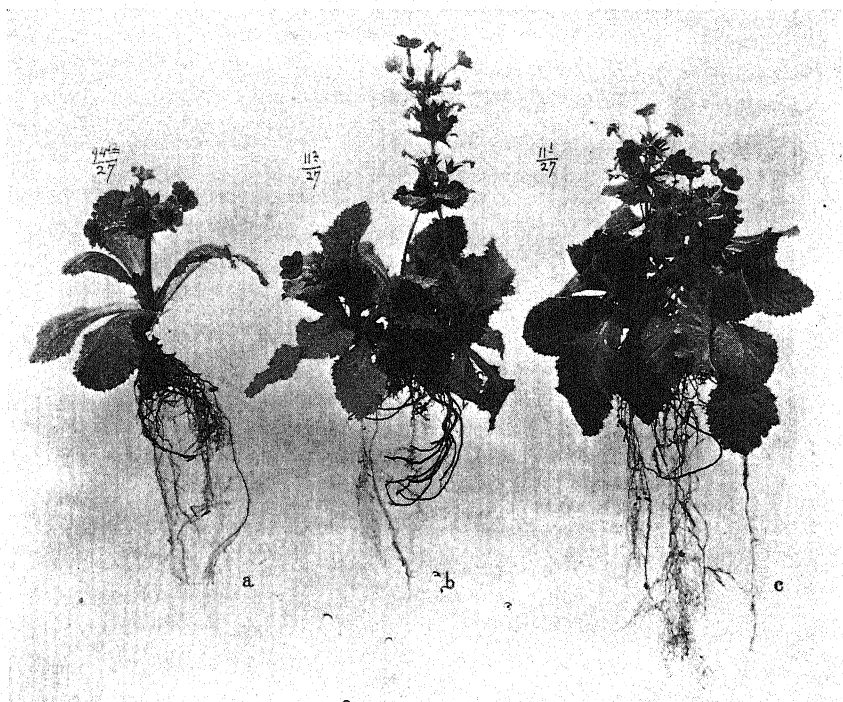
Text-fig. 12. (a) long-styled plant; (b) mealy plant; and (c) plant of type B, of equal ages, showing the comparatively small amount of growth made by the long-styled plant. (The roots of (b) were actually as long as those of (c) but had been broken off.)

(Plate XIII, fig. 15). The young leaves are often recurved and the irregularity may increase with age. Full grown stems and leaves are tough and difficult to break, and after cutting they retain their stiffness and apparent turgidity for a long period as compared with those of other types. The formation of secondary crowns is much retarded or even inhibited, for in some plants sub-terminal growth has persisted into the second season, only a single crown being formed. In Text-fig. 12 such a plant is shown ($94^{12}/27$) together with one of normal growth

(11²/27), and one of type B (11¹/27). In this figure the three plants are all in their second flowering season and approximately of the same age. It illustrates the comparative modes of development characteristic of the types B and D. The plant 94¹² is small, but reduction in size is not an essential characteristic of this type. The dark foliage[1], a feature often associated with dwarfness (*e.g. Campanula persicifolia* var. *nitida* A. E. Gairdner, 1926, and *Cochlearia danica* M. B. Crane and A. E. Gairdner, 1923) is possibly a constant feature. A curious abnormality occasionally observed when the plant is about to flower is the elongation of the stem below the first leaves (Text-fig. 13).
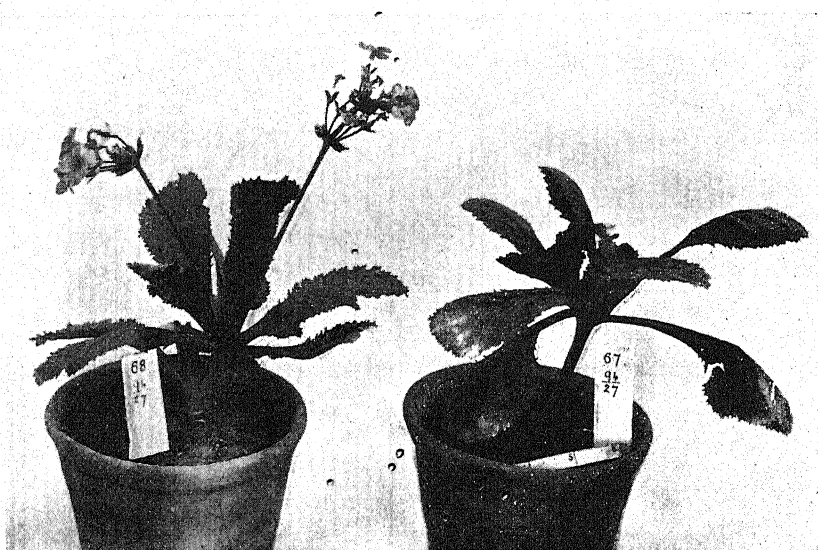
Text-fig. 13. Homo-styled (no. 68) and long-styled (no. 67) plants in family 94/27, showing the elongated stem occasionally seen in plants of type D. The difference between the leaves of homo- and long-styled plants of this family is also shown.

The homo-styled plants are exceedingly variable in habit. Among them are forms not markedly aberrant, but with one or other characteristic of the family, *e.g.* large foliage, reduced mealiness or pouched

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[1] Mr E. Phillis has examined alcoholic chlorophyll solutions of this type and of type B, but found no difference between them. Miss E. Philip Smith has made a preliminary examination of the anatomy of the two forms, and finds that in the "long-styled" plants, "the midribs of the leaf have more numerous vascular strands than those of the B type (about 9 to 5) but when the actual number of lignified elements was counted there was found to be very little difference between the two. The greater dissection of the wood in the 'long-styled' leaf would account for the greater difficulty in tearing compared to the leaf of B type."

TABLE II.

Plants of type D.

I. 11/25 self-fertilised (chromosomes not counted).
II–IV. Families from plants in I.
II. Long-styled plants self-fertilised.
III. Homo-styled plants self-fertilised.
IV. Crosses between homo- and long-styled plants.

	Parent	Family	Number of capsules	Progeny Homo-styled	Progeny Long-styled	Habit	Chromosomes	Parents of families in II–IV
I	$11^p/25$	58/26	1 (about 80 seeds)	27		21 homo-styled	13 uncounted	75/26
							5 with 36	72/26, 74/26, 94/26
							3 with 37	93/26
					5 long-styled	2 with 36	54/26, 71/26
							1 with 35	95/26
							1 with 37	86/26
						1 peculiar, mealy	1 with 36	49/26
					11	8 long-styled	5 uncounted	46/26, 47/26
							2 with 37	59/27
							1 with 35	52/26
						3 approach long-styled	2 uncounted	50/26, 51/26
							1 with 36	55/26
II	$52^p/26$	81/27	2 (1 with few seeds)		24	All long-styled	2 with 36	66/27
							2 with 35	68/27
	$46^p/26$	90/27	2		78*	Long-styled except a few	1 with 35	
							1 with 36	
							1 with 37	
	$47^p/26$	91/27	1		71	All long-styled		
	$50^p/26$	92/27	2		133*	Many types, few long-styled		
	$51^p/26$	93/27	2		105	Many types, few long-styled		
	$55^p/26$	87/27	2	1*	114	Many types including long-styled		
III	$54^p/26$	94/27	2	57	—	Approach long-styled		
					19	Long-styled		
	$71^p/26$	100/27	2	5	—	1 plant long-styled		
					1	Long-styled		
	$72^p/26$	101/27	2	24	3	Many types, a few long-styled		
					3	+many discarded as seedlings		
	$74^p/26$	102/27	2	69	—	Many types, a few approach long-styled		
	$75^p/26$	103/27	2	27	13	Many types, a few long-styled		
	$86^p/26$	104/27	2	48	11	Many types, a few long-styled		
	$93^p/26$	105/27	2	43	10	Many types, a few long-styled		
	$94^p/26$	106/27	3	37	—	Many types, a few long-styled +17 plants discarded		
	$95^p/26$	107/27 & 19, etc./28	Several	56	—	Approach long-styled		
					3	Long-styled	1 with 36	
	$49^p/26$	12/28 & 30/28	9 (about 30 seeds)	14	—	Peculiar, various grades of mealiness		
IV	$52^p/26 \times 19/25^p$	82/27	2 (1 with few)	24	—	A few approach long-styled		
	$49^p/26 \times 46^p/26$	85/27	1 (about 15 seeds)	4	—	None long-styled		
	$49^p/26 \times 51^p/26$	86/27	2 (5 seeds, ? good)	2	—	Neither long-styled		
	$55^p/26\dagger \times 71/26$	88/27	1	7	—	Some approach long-styled		
					2	1 plant long-styled		
	$55^p/26 \times 86/26$	89/27	1	20	15	A few long-styled		
	$41^p/26\ddagger \times 46^p/26$	95/27	1	32	—	None long-styled +some discarded as seedlings		
	$46^p/26 \times 41^p/26$	96/27	1	22	—	1 plant approaches long-style		

* A few have very short styles but the stamens low.

† When harvesting the seed of 55/26 it was noted that a few seeds from the crossed capsule 55/ ×71/26 had possibly dropped among those of 55/26 self-fertilised. The single homo-styled plant is presumably due to this accident.

‡ The plant 41/26 was a normal tetraploid (chromosomes not counted).

anther sacs. They grade to extremely abnormal forms with very large leaves, much buckled and distorted by hypertrophied growth. One of the most normal plants is shown in Text-fig. 11, *A* (58 A/26); nevertheless, this plant may be recognised as belonging to the family by its large full leaves. The two plants *A* and *C* in this figure show the type of growth commonly associated with the homo- and long style respectively, neither being an extreme example.

Both among homo- and long-styled plants were a few with the habit common to the opposite form, or at least with some of its features. The significance of these plants is not as yet understood, but they are doubtless of importance in the genetical analysis of this type. The obvious suggestion that the different habits are due to factors independent of, but linked with, those for heterostylism, is probably right, but further analysis is required to establish such a linkage (or linkages). The plants are extremely difficult to record, owing to the numerous intergrading forms. Many of these are doubtless due to differences in number of chromosomes rather than qualitative difference.

A single individual of the homo-styled group, with 36 chromosomes, was of exceptional interest (no. 49/26, Text-fig. 11, *B*). In general habit the plant was not unlike its homo-styled sister plants which show traces of the long-styled habit, but it differed in that the margin of the leaf was flatter and the teeth symmetrical, patches of meal were distributed irregularly, the sepals were small with long pointed limbs. The plant was highly sterile both as a male and female. The stigma was abnormally large, and very little pollen was produced. In the combination of mealiness with flat tapering leaves and fine pointed sepals the plant was more like *verticillata* than any other tetraploid plant we have seen. By self-fertilisation of many flowers we succeeded in raising from it 14 plants, all homo-styled. Among them are mealy plants with very little pollen, showing peculiarities in the distribution of meal. The investigation of these plants may throw light on the constitution of their parent.

Although it is usually easy to discriminate between equal and long-styled plants, yet in each group there is great variability, *e.g.* in the relative position of anthers and stigma, in length of style and of corolla tube, and in the structure of the tube, whether pouched at the anthers or tapering. Occasionally plants are not easily classified by the position of their anthers in relation to the stigma. Such plants have however always proved to be clearly differentiated in the size of their pollen. That this should be so is surprising in view of the fact that in this section no plant has been found with uniform pollen. In every plant examined

a considerable though varying number of shrivelled grains has been found, and even the grains capable of swelling in water show great differences in size. This range in size makes it difficult to obtain reliable measurements, and those given in Table 5 (p. 464), are only of comparative value. The same objection applies in some degree to other tetraploid pollen measurements, and especially to those of type C, of which type only two plants, each with 35 chromosomes and consequently with much defective pollen, were available for measuring.

The average size of the pollen of individual plants of the parent species and of various tetraploids is fairly uniform, ranging from 25–29μ in homo-styled plants, and from 19–25μ in long-styled plants. Considering the small number of grains measured, and the complexity of the material, these differences are slight. The range in a single plant of *floribunda* may be 5μ, and of *verticillata* 4μ. We had no long-styled *floribunda* to compare with the homo-styled, but two comparable long-styled *isabellina* varieties were available. The average size of their pollens is 19 and $21\cdot5\mu$ respectively. The difference is probably real, for the pollen of these varieties is fairly uniform, but similar differences between individual tetraploids are of doubtful significance, and they have accordingly been tabulated together except in a few cases. In general, the pollen of homo-styled tetraploids with little defective pollen is similar in size to that of *verticillata*, and of homo-styled *floribunda*, but the pollen of those with much defective pollen is a little smaller. This difference is possibly due to the measurement of occasional defective grains indistinguishable from sound grains. On the other hand pollen of long-styled tetraploids is slightly larger than that of the two long-styled varieties of *floribunda* measured, in spite of the fact that such tetraploids have much defective pollen.

The breeding experiments are set out in Table II. Between the homo- and long-styled condition the relation appears to be simple. Six long-styled plants, 1 with 35 chromosomes, 1 with 36, and four uncounted, bred true on self-fertilisation. Nine homo-styled plants gave as follows:

2 with 36 chromosomes gave only homo-styled, 69 and 37 plants respectively.
2 with 36 chromosomes gave homo- and long-styled in the ratio of 3 : 1 or approximately that ratio; in addition, one gave a family of 6 plants, 5 homo- and 1 long-styled. (81:25).
2 with 37 chromosomes gave homo- and long-styled in the ratio of 4 : 1. (91:21).
1 with 35 chromosomes gave homo- and long-styled in the ratio of 19 : 1. (56:3).
1, chromosomes uncounted, gave homo- and long-styled in the ratio 2 : 1. (27:13).

The total, including the parent family (27 : 11) but omitting the progeny of $95^p/26$ (56 : 3), is 231 homo-: 71 long-styled, or in the ratio

3 : 1, as would be expected from tetraploid plants having one "dose" of the dominant factor. The constitution of the two plants giving homo-styled offspring only, and of $95^p/26$, is doubtful and requires further analysis.

From the crosses but little information is obtained, except the fact that long-styled plants crossed with true-breeding homo-styled plants give only homo-styled offspring.

Summary of experiments on tetraploids with aberrant chromosome numbers.

The results of breeding the three original plants of types B, C and D are in agreement in that normal tetraploids were not found among their offspring. In each group certain characteristic features of the parent plant reappear, irrespective of the number of chromosomes. In the case of type B the evidence is not extensive, but from types C and D some 3000 plants were grown in the second generation and forms common to both groups were not observed. Some of the features by which the three groups are distinguished are evasive and difficult to describe, but the general difference in appearance is very striking. Most conspicuous in the case of type D, was the absence of individuals with the corolla tube as long as in the normal tetraploid; in type B, plants with flowers of the full (normal) yellow are missing, and in type C, the foliage and general habit are perfectly distinct from those in B and C.

The absence or rarity of "balanced" tetraploids with complete sets of *floribunda* and *verticillata* chromosomes would be explained if the original loss of a chromosome followed upon interspecific pairing and interchange. Thus, if pairing is V_1–F_1, V_1–F_1 instead of V_1–V_1, F_1–F_1, and one of the set is subsequently lost, the resulting gametes would contain one, or two, modified chromosomes of the set. Fertilisation of the gamete containing the one (represented below by F_1') by a normal tetraploid gamete, would give a zygote of the constitution $F_1V_1F_1'$. If in such a zygote the pairing is intraspecific, four types of gamete would be formed in equal numbers, F_1V_1, $F_1'V_1$, F_1' and F_1. The chances of the F_1V_1 gametes being normal (*i.e.* complete in respect of *verticillata* and *floribunda* factors) would depend upon the number of differences and on the amount of crossing over between the F_1 and F_1' chromosomes.

But if, as a result of the original interspecific pairing, intraspecific pairing in the homologous set is less prevalent than in the normal tetraploid, then the chance of obtaining a normal gamete is still smaller.

3. Crosses between the Tetraploid hybrid and Diploids.

In this section a preliminary report will be made on the 3n and 4n plants recently obtained from crosses of *floribunda* and its varieties with tetraploids of type *D*. An account will also be given of the 26-chromosome plant 18/12, for although we have no proof that this plant is derived from a triploid, yet its genetical and cytological behaviour is of the triploid type.

The plant 18/12 has already been described in some detail (Pellew and Durham, pp. 171–175). Its history is briefly as follows: In 1911 Messrs Veitch sent to us five plants purporting to be "maternal hybrids" derived from *kewensis 4n × floribunda*. Apparently they resembled tetraploid plants obtained from Kew at the same time. Four were self-fertilised; the seed of only one germinated giving two plants of which one died young and the other was the exceptional plant numbered 18/12, since found to have 26 chromosomes.

In view of these facts we incline to regard the five plants sent us by Messrs Veitch as triploids (or possibly tetraploids arising from unreduced egg-cells). Although we have failed to obtain triploids except when we have used plants of type *D* as the tetraploid parent, it is possible that in Messrs Veitch's crosses some other aberrant tetraploid type was used, capable of giving triploids. Evidently their plants did not resemble our own triploids which are thickly covered with long glandular hairs and hence could not have been mistaken for tetraploids of the normal type. In addition, a group of plants sent to us by Veitch at the same time from *kewensis 4n × floribunda* var. *isabellina* (Pellew and Durham, p. 170) appear to have been a mixture of tetraploids and triploids. We therefore suggest that Messrs Veitch succeeded in raising triploids which resembled normal tetraploids.

The plant 18/12 approaches *kewensis 2n* in general appearance, but the leaves are shorter and broader, and the whole plant less vigorous (Text-figs. 16–17). It is very slightly hairy, the hairs on the young leaves being visible without magnification, and it has a ring of meal in the calyx. The anthers are often shrivelled; when pollen is produced about 40 per cent. of the grains appear sound. The diameter of the good pollen grains is about 25μ. It is almost sterile as a female. As a pollen parent it is in some degree fertile on *floribunda* and its varieties, and also, in less degree, on *kewensis 4n*. We have recently repeated these crosses and investigated the cytology of many of the progeny.

Kewensis tetraploid × 18/12.

This cross rarely gives seed, but we have succeeded in making it twice, 2 capsules giving 1 plant and 22 plants respectively. As to the F_1 plants we have little to add to the statement made on p. 421. They vary but slightly from known tetraploid types. One with 34 chromosomes
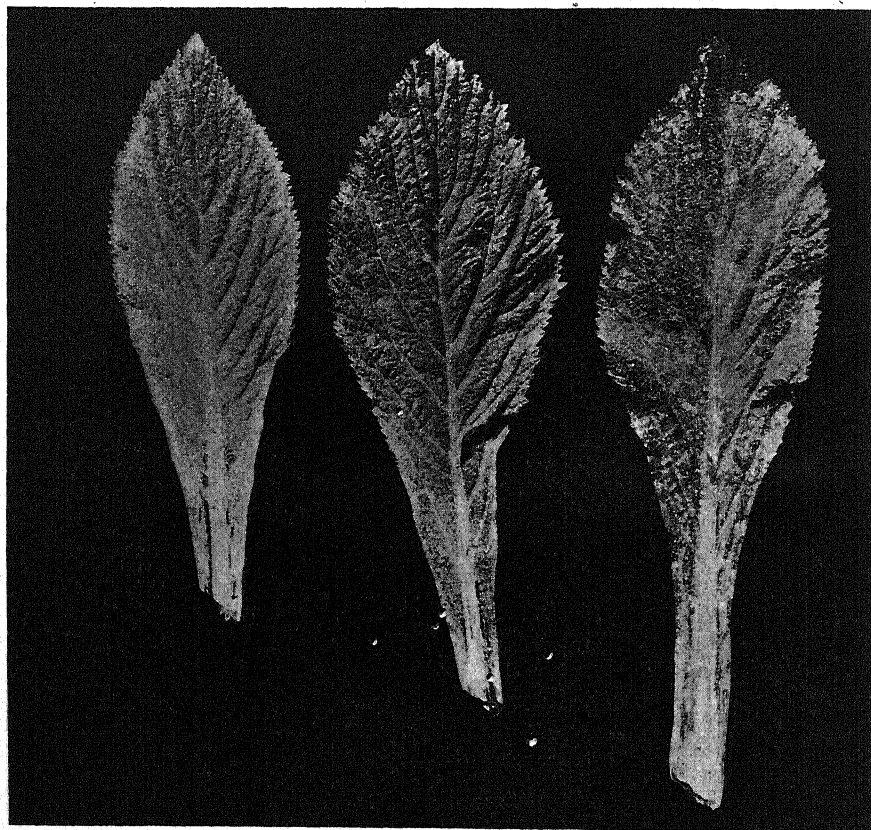
Text-fig. 14. *Primula verticillata.* Leaves at different stages of development.

scarcely differed from a plant of type C, having the flat rosette common in such plants. This 34-chromosome plant was in some degree fertile, and thus differs strikingly from the abnormal and sterile 34-chromosome plants of tetraploid types B and C. Presumably this plant lacks two different chromosomes, whereas the sterile forms lack two of the same kind.

It appears that in this cross the effective gametes of 18/12 approximate to those of a normal tetraploid.

Floribunda × 18/12 (Pellew and Durham, p. 172).

The capsules may contain as many as 70 seeds, varying in size, of which about a third may germinate. (*Floribunda* capsules may contain 400 seeds.) The F_1 families are extremely heterogeneous, showing variability in every character. Dwarf plants, with leaves 2 in. long and the
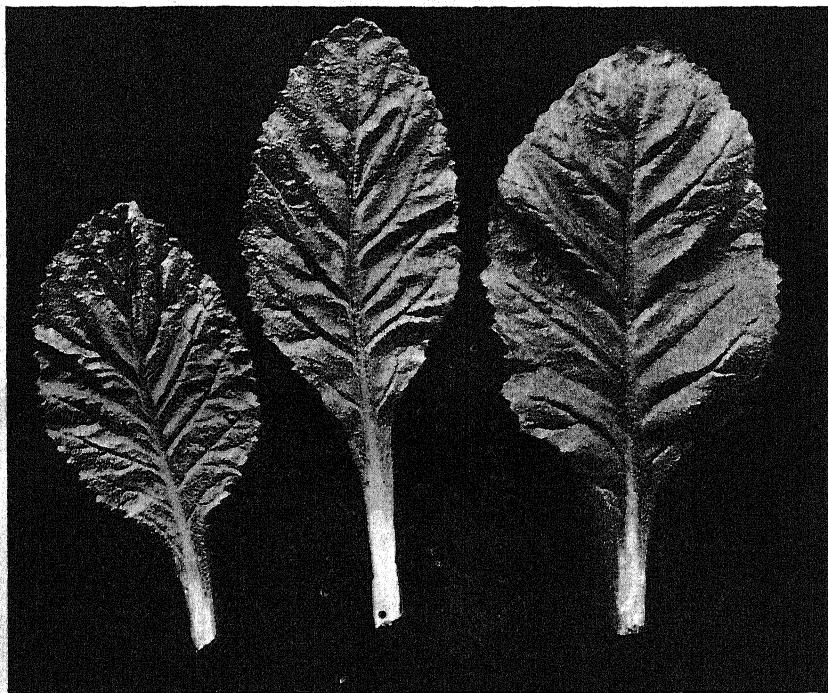
Text-fig. 15. *Primula floribunda.* Leaves at different stages of development.

first tier of flowers about 1 in. from the stock, grade to large plants with leaves 6 in. long and the first tier of flowers at a height of 4 in. Short broadly ovate leaves grade to long narrow leaves with winged petioles. The base of the lamina may be tapering or truncate, the tip pointed or obtuse, the margin serrate or dentate. Growth may be irregular, the leaves incurved or extremely rugose with frilled margins. The surface grades from a thick covering of long hairs to a glabrous shiny surface. Long- and homo-styled plants appear in numbers approaching normal Mendelian ratios, and also occasional intermediate forms not

easily assigned to either class. In flower colour the plants grade from almost pure white to a very deep yellow, and in size, shape and texture of the flowers they are exceedingly variable.

In view of the extreme heterogeneity of these plants it is surprising to find among them a high degree of fertility. In some there is little
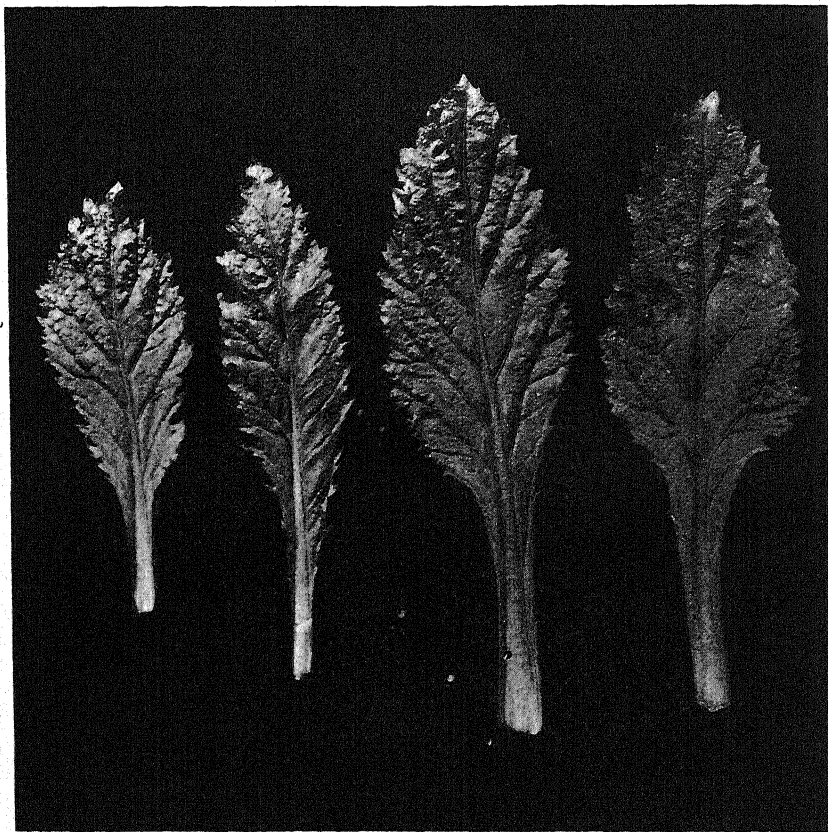
Text-fig. 16. Leaves of 18/12 at different stages of development.

pollen and such plants may fail to set seed on self-fertilisation, but fertilised with *floribunda* pollen they have generally proved fertile.

The main classes are shown in Table III. The numbers given of long- and homo-styled, yellow and pale flowered plants are probably accurate, but those for long and short hairs, and for broad, ovate and narrow leaves, represent only the general constitution of the families in these

characters. These last characters are often exhibited by plants with 19 or more chromosomes. The families are set out according to the constitution of the diploid mother, *i.e.* homozygous homo-styled or long-styled, and homozygous or heterozygous yellow. In the last family (26/26) the mother was a long-styled cream-coloured plant actually derived from *floribunda*× 18/12.
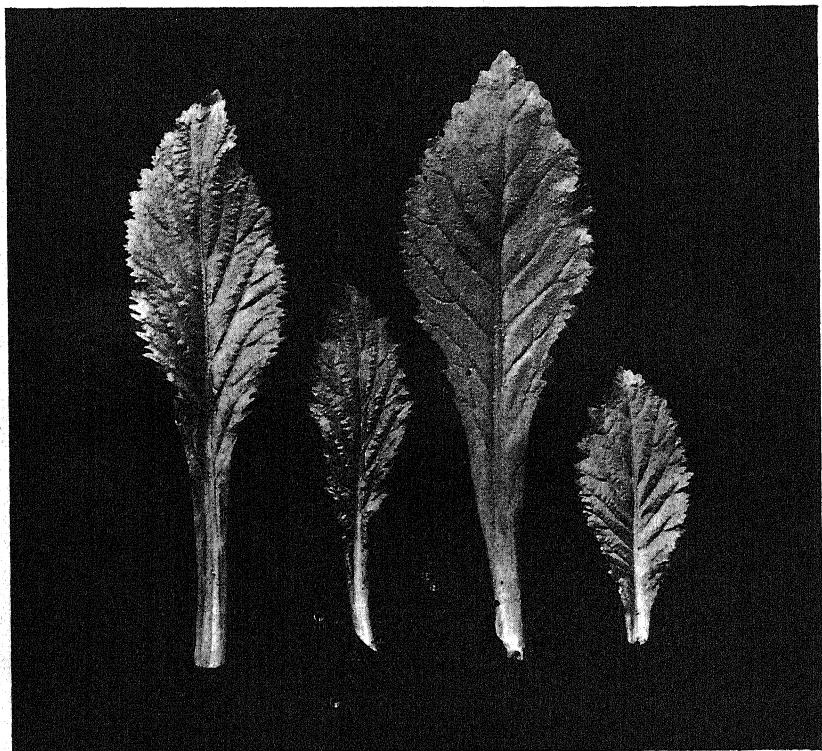
Text-fig. 17. Young and mature leaves of *kewensis* 2n, and of a plant from the cross *floribunda* × *kewensis* 2n (on the right).

In all the families from homo-styled mothers plants with long hairs and ovate leaves are the most common (both these characters being derived from *floribunda*). But from long-styled mothers, short haired plants are almost as numerous as long haired, the total numbers being 28 long : 22 short haired, and from homo-styled mothers, 141 long : 22 short haired. Among the short haired plants the surface may be shiny, or dull as in *floribunda*. Moreover, in 26/26 (from cream long-styled

mother) the chromosome numbers of the short haired plants examined are higher than those of similar plants in 24/25 and 27/26, etc. (from homo-styled mothers) the numbers being 4 plants with 19 chromosomes as compared with 1 with 19, 2 with 20 and 3 with 21 chromosomes. Hence

TABLE III.

Families from floribunda × 18/12.

Female parent	Seed number	Flower structure		Flower colour		Hairs		Leaves		
		Long style	Homo-style	Yellow various	White and cream	Long	Short	Broad	Ovate	Narrow
Floribunda										
Yellow homozygous.	124/14	—	14	14	—	13	1	2	12	—
Homo-style homozygous	24/25 etc. ⎫ 27/26 etc. ⎭	—	101	101	—	89	12	16	70	15
Floribunda										
Yellow homozygous. Long style	220/13 ⎫ 361/13 ⎭	16	10	26	—	13	13	8	17	1
Floribunda										
Yellow heterozygous.	196/13	—	7	5	2	—	—	—	—	—
Homo-style homozygous	338/13	—	28	28	—	—	—	—	—	—
	100/14 ⎫ 140/14 ⎭	—	48	47	1	39	9	6	39	3
Ex floribunda × 18/12										
Cream, long style	26/26	10	14	28*	—	15	9	2	20	2

* Including two plants lemon-yellow, the pigment evenly distributed.

Chromosome numbers in 24/25 and 27/26, etc.

Hairs long.	Leaves broad	1	with 18	chromosomes	
,,	,,	1	,,	20	,,
,,	ovate	3	,,	18	,,
,,	narrow	1	,,	18	,,
,,	,,	4	,,	19	,,
,,	,,	1	,,	20	,,
Hairs short.	Leaves broad	2	,,	19	,,
,,	ovate	2	,,	19	,,

Chromosome numbers in 26/26.

Hairs long.	Leaves ovate	1	with 18	chromosomes	
,,	ovate-flat	1	,,	20	,,
,,	narrow	1	,,	18	,,
Hairs short.	Leaves broad	1	,,	21	,,
,,	ovate	1	,,	19	,,
,,	,,	1	,,	20	,,
,,	ovate, peculiar	1	,,	20	,,
,,	narrow	2	,,	21	,,

the larger number of short haired plants appears to be the consequence of a greater fertility of male gametes bearing 11 and 12 chromosomes on long- than on homo-styled plants.

We have little evidence bearing on the nature of the association between short hairs and high chromosome number, nor on the com-

paratively high fertility of this combination on long style. The F_1 records show clearly that in the various classes in respect of hairiness and surface, homo- or long-styled plants may occur, even when the homostyle factor is known to come from 18/12 (*e.g.* in Nos. 220 and 361/13, and in 26/26). A number of F_2 families from short haired shiny plants were raised in 1913 and 1914. They contained a great mixture in respect of hairiness but the majority were long haired with dull surface (as in *floribunda*) and the minority short haired, generally with shiny surface. When homo- and long-styled plants were present in these families they were distributed, approximately as 1 in 4, alike among the long and short haired plants. The point is of interest not only in relation to the different classes produced when 18/12 is crossed on to long-styled plants, but also because in certain long-styled triploids to be described below, a definite association between hairiness and the heterostyle factors is indicated. No way of reconciling these cases with the genetical behaviour of 18/12 can at present be found, but in neither has the analysis been carried very far.

Another character of 18/12 derived from *verticillata* is a long narrow leaf tapering to the petiole. Among the progeny of *floribunda* × 18/12 plants with leaves of this type but longer and narrower than those of 18/12 have been found to have 19 or more chromosomes (*e.g.* $24^1/25$, Fig. 18) but two, with leaves of a less extreme type, were found to have 18 chromosomes. On self-fertilisation the narrow leaved plants give a majority of broad leaved forms, the parental type appearing but rarely. The following account of the results of breeding from the narrow leaved 19-chromosome plant $24^1/25$ is typical of several families.

Plant $24^1/25$. Long narrow leaves (Fig. 18). Fully hairy. Homostyle.

Two flowers self-fertilised gave about 450 seeds, varying in size, sown under numbers 35 and 36/26. About 120 seeds germinated from which 96 plants were raised. They varied in size, habit, shade of green, leaf shape, length of hairs (none were short haired).

The following records were made:

68 homo-styled	9 narrow leaved	
		1 with 18 chromosomes ($36^1/26$ see below)
		2 with 19 chromosomes
	59 broad leaved	
28 long-styled	4 narrow leaved	
	24 broad leaved	
Total	96	

The narrow leaved plant with 18 chromosomes, $36^1/26$ (Fig. 17), was bred from, 107 plants being raised by self-fertilisation, of which only

13 approached the parent in leaf shape, the rest being definitely broader leaved. These plants were not examined cytologically.

One other narrow leaved plant of known chromosome number, a sister plant of $24^1/25$, was bred from, as follows:

Plant $24^3/25$, long narrow leaves (Fig. 19). Fully hairy. Pollen good, few grains shrivelled. Leaf surface exceptionally flat, and the margin very broadly toothed.
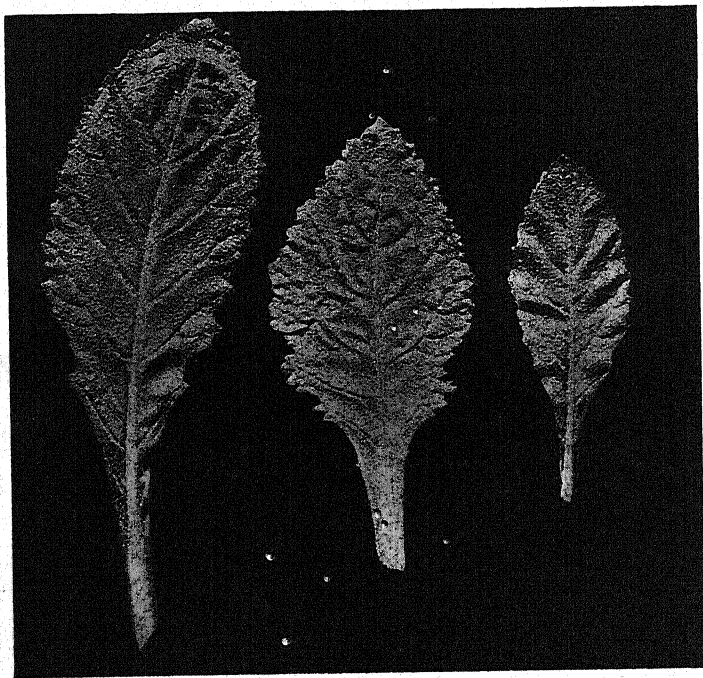

Text-fig. 18. Leaves of $24^1/25$, a 19-chromosome plant (on the left) and of two of its progeny, 36^1, 18 chromosomes and 36^2, 19 chromosomes (in the centre).

Two flowers self-fertilised gave about 500 seeds, grading from very small to full size, sown under numbers 32 and 33/26. About 130 germinated of which 112 plants were grown to maturity. They varied in leaf shape, habit, size, etc. The following classes were counted:

88 homo-styled	5 approached the parent in leaf shape, surface or margin, the rest being broader leaved. Three with 19 chromosomes (of which 2 were very small plants, one = 32^{14}, Fig. 19)
	57 normal sized plants, 2 with 18 chromosomes, (one = 32^{12}, Fig. 18)
	26 small plants, 2 with 18 chromosomes
24 long-styled	17 normal sized plants
	7 small plants

Total 113

Among the many new shades of yellow and cream which appeared in F_1 and F_2 from *floribunda* × 18/12 were some picotee forms, the ground colour being intermediate between the full yellow of *floribunda* and the variety *isabellina*, with the margin and "eye" of a darker shade. These forms (originally called "lemons") were found to be recessive to yellow and dominant to *isabellina*. All three types might be given by yellows. Later it was found that lemon × *isabellina* gave lemon (or lemon and *isabellina*) if the *isabellina* parent came from a lemon, but

Text-fig. 19. Leaves of 24³/25, a 19-chromosome plant on the left, and of two of its progeny 32¹², 18 chromosomes and 32¹⁴, 19 chromosomes on the right.

if it came from a yellow, then yellow offspring might also be produced by this cross. Hence it appeared that two factors were involved, one for yellow (**Y**) epistatic to a "lemon" factor (**L**); the absence of **L**, or of **L** and **Y**, giving the two kinds of *isabellina* (**Yl** and **yl**). On this scheme the old variety *isabellina* is **Yl**, for crossed with yellow it gives in F_2 only yellow and *isabellina*, and crossed with lemon it gives F_1, yellow and F_2, yellow, lemon and *isabellina* in ratios approximating to $9:3:4$. The ratios are not always in complete agreement with the scheme, but some

of the irregularities, especially in the early experiments, may have been connected with the presence of extra chromosomes.

The possibility that **Y** and **L** are linked, or that either is linked with heterostylism, has been tested. These tests were made with plants derived from crosses of *isabellina* and lemon with *floribunda* (Table IV).

Summary of experiments on 18/12 (26 *chromosomes*).

The pollen of 18/12 is to some degree fertile on:

(*a*) *kewensis* 4*n*. The offspring approximate closely to the tetraploid parent in appearance, chromosome number and breeding.

(*b*) *floribunda* homo-styled. The offspring are exceedingly diverse but generally have the diploid number of chromosomes. A small number possess well marked *verticillata* characters and these plants usually have one or two chromosomes in excess of the diploid number.

(*c*) *floribunda* long style, and the var. *isabellina* long style (ex *floribunda* × 18/12). The offspring are even more diverse than in (*b*). Plants with *verticillata* characters occur more frequently, and they may have as many as three chromosomes in excess of the diploid number.

TRIPLOIDS, 1927.

Five plants were obtained from crosses of diploids with *kewensis* 4*n* of type *D* (p. 438). The details of the crosses are as follows:

(1) 6 × 87/26, *isabellina* long style (**Yle**) × *kewensis* long style (35 or 36 chromosomes).

Seed from 2 capsules sown, in each some good seed.

Two seeds germinated, giving plant no. 11/27, 35 or 36 chromosomes, and plant no. 22/27, 27 chromosomes.

(2) 33 × 86/26, *isabellina* long style (**yle**) × *kewensis* homo-style, giving homo- and long style (37 chromosomes.)

Seed from four capsules sown, a few good seeds in each.

One seed germinated, giving plant no. 16/27, long style, 28 chromosomes.

(3) 47 × 39/26, *kewensis* 4*n* long style × lemon homo-style (**yLE**).

Seed from 1 capsule sown, no good seed, a few "doubtful."

One seed germinated, giving plant no. 34/27, homo-style, chromosomes not counted.

(4) 50 × 2/26, *kewensis* 4*n* long style × *isabellina* long style (**yle**).

Seed from 1 capsule sown.

One seed germinated, giving plant 60/27. Chromosomes not counted.

Of these 5 plants, the 4 with long styles are self-fertile, and the single homo-styled plant (34/27) is sterile as a female. All have long hairs, but those of 34/27 are of a maximum length of 4–5 cells, while those of the long-styled may be 12–14 cells long. From the three plants 16, 22 and 11/27 families have been raised by self-fertilisation. They contain a profusion of forms, generally of a large and robust type. The progenies of the "triploid" and "tetraploid" parents are indistinguishable in size or general habit, and moreover the chromosome numbers appear to be approximately tetraploid in both. Perhaps the most significant feature of these families is the complete absence of plants with the glabrous surface of *kewensis* $4n$. All (280) are long haired, in spite of the (presumed) introduction of the glabrous character by the $4n$ parent. Several *verticillata* characters are evident; no plant appears to be without meal in the calyx, although the amount varies considerably, and many are scented. To account for the absence of glabrous plants it may be suggested that the original tetraploid gametes introduced into the triploids were abnormal, having lost the glabrous factor. This supposition might also explain the peculiar characters of the triploids, their fertility, hairiness, etc. But the suggestion is scarcely justified at this early stage of the inquiry.

The only difference found between the progenies of "triploids" and "tetraploids" is in the ratio of yellow : *isabellina* plants. A few picotee ("lemon") plants also appear, but of these the numbers recorded are of more doubtful significance for they grade to a pale self-lemon class (? $\mathbf{yL}$) in which the picotee factor may be masked, and which are difficult to separate from the pale shades of yellow ($\mathbf{YL}$). The records are as follows:

Ex 11/27 (35 or 36 chromosomes) from $2n$ parent $\mathbf{Yl}$;

Offspring:	Yellow	Lemon "picotee"	Isabellina
	171	1	5

Ex 22/27 (27 chromosomes) from $2n$ parent $\mathbf{Yl}$;

Offspring:	Yellow	Lemon "picotee"	Isabellina
	55	2	3

Ex 16/27 (28 chromosomes) from $2n$ parent $\mathbf{yl}$;

Offspring:	Yellow (many pale)	Lemon "picotee"	Isabellina
	36	4	3

It will be noticed that the *isabellina* plants from 11/27 are about as 1 in 36, the normal tetraploid ratio from a double heterozygote ($\mathbf{L_2 l_2}$).

TABLE IV.

E, homostyle.   e, long style.
YL, yellow.   yL, picotee ("lemon").
Yl, *isabellina*.   yl, *isabellina* (pale).
S.-fd = self-fertilised.

Type and parent numbers	Origin of parents*	Factors	Observed Y E	Observed Y e	Observed L E	Observed L e	Observed l E	Observed l e	Expectation Y E	Expectation Y e	Expectation L E	Expectation L e	Expectation l E	Expectation l e
Yellow YyL														
1.17	I	YyLE s.-fd	143	—	29	—	—	—	129.0	—	43.0	—	—	—
7.17	I	YyLE s.-fd	141	—	43	—	—	—	138.0	—	46.0	—	—	—
10.18	I	YyLE s.-fd	24	—	12	—	—	—	27.0	—	9.0	—	—	—
29.17	I (a)	YyLEe s.-fd	45	22	21	6	—	—	53.1	17.7	17.7	5.9	—	—
Yellow YLl														
45.13	II	YLlEe s.-fd	20	6	—	—	3	3	18.0	6.0	—	—	6.0	2.0
46.13	II	YLlEe s.-fd	14	2	—	—	4	1	10.8	3.6	—	—	3.6	1.2
50.13	II	YLlEe s.-fd	18	5	—	—	9	—	18.0	6.0	—	—	6.0	2.0
54.13	II	YLlEe s.-fd	12	4	—	—	8	7	18.0	6.0	—	—	6.0	2.0
4.13	II	YLlE s.-fd	18	—	—	—	7	—	18.6	—	—	—	6.2	—
49.13 × 4.13	II & II (a)	Yle × YLlE	16	—	—	—	19	3	17.5	—	—	—	17.5	—
5.13	II	YLlE s.-fd	29	—	—	—	17	1	23.0	—	—	—	23.0	—
5.13 × 49.13	II & II (a)	YLlE × Yle	15	—	—	—	23	7	19.0	—	—	—	19.0	—
49.13 × 5.13	II & II (a)	Yle × YLlE	15	—	—	—	27	—	21.0	—	—	—	21.0	—
Yellow YyLl														
6.21	III	YyLlEe s.-fd	32	20	14	2	13	3	36.0	12.0	12.0	4.0	15.0	5.0
6.21 × 8.21	III & VII	YyLlEe × yle	4	7	2	4	4	7	3.5	3.5	3.5	3.5	7.0	7.0
8.21 × 6.21	VII & III	yle × YyLlEe	4	2	4	3	5	6	3.0	3.0	3.0	3.0	6.0	6.0
7.21	III	YyLlEe s.-fd	48	14	19	6	20	5	48.0	16.0	15.6	5.2	21.0	7.0

Continuation of table (column headers appear on the preceding page).

Cross	Group	Mating												
7.21 × 8.21	III & VII	YyLlEe × yle	8·0	8·0	4·0	4·0	4·0	4·0	9	5	4	4	3	6
8.21 × 7.21	VII & III	yle × YyLlEe	6·6	6·6	3·3	3·3	3·3	3·3	3	9	3	2	3	7
34.21	III	YyLlE s.-fd	—	11·2	—	8·4	—	25·2	—	8	—	8	—	29
35.21 × 34.21	VII & III	yle × YyLlE	—	25·0	—	12·5	—	12·5	—	28	—	12	—	10
9.21	IV	YyLlE s.-fd	—	13·5	—	18·0	—	40·5	—	20	—	9	—	43
8.21 × 9.21	VII & IV	yle × YyLlE	—	10·5	—	5·2	—	5·2	—	8	—	8	—	5
18.21	IV	YyLlE s.-fd	—	8·0	—	6·0	—	18·0	—	3	—	3	—	26
12.21	V	YyLlEe s.-fd	3·0	9·0	2·0	6·0	6·0	18·0	2	14	1	4	5	20
23.21 × 12.21	VII & V	yle × YyLlEe	5·5	5·5	2·7	2·7	2·7	2·7	4	10	—	5	—	4
11.21	VI	YyLlEe s.-fd	4·8	14·4	3·6	10·8	10·8	32·4	8	11	2	6	15	38
11.21 × 10.21	VI & VIII	YyLlEe × ylEe	1·5	4·5	1·1	3·3	3·3	9·9	2	51	21	7	—	5
10.21 × 11.21	VIII & VI	ylEe × YyLlEe	1·4	4·2	1·0	3·0	3·0	9·0	1	19	4	25	3	5
22.21 × 8.21	VI & VII	YyLlEe × yle	40·0	40·0	22·5	22·5	22·5	22·5	43	8	2	—	16	24
8.21 × 22.21	VII & VI	yle × YyLlEe	12·2	12·2	6·1	6·1	6·1	6·1	18	—	—	3	2	6
35.21 × 42.21	VII & VI	ylE × YyLlEe	5·5	5·5	2·7	2·7	2·7	2·7	4	—	—	—	3	2

Lemon yLl

Cross	Group	Mating								
6.20	VI	ylLEe s.-fd	1·7	5·1	5·1	15·3	3	6	2	16
6.20 × 1.20	VI & VIII	ylLEe × Yle	3·5	3·5	3·5	3·5	8	1	3	2
1.20 × 6.20	VIII & VI	Yle × YyLlEe	8·0	8·0	8·0	8·0	7	8	6	11
16.20	VIII	ylLIE s.-fd	1·5	4·5	4·5	13·5	1	7	4	13
5.19	IX	ylLIE s.-fd	—	7·3	—	21·7	—	7	—	22
5.19 × 25.19	IX	ylLIE × ylE	—	12·0	—	12·0	—	8	—	16
7.19	IX	ylLIE s.-fd	—	5·5	—	16·5	—	6	—	16
7.19 × 1.19	IX	ylLIE × ylE	—	15·0	—	15·0	—	16	—	14
7.19 × 11.19	IX	ylLIE × yle	—	15·0	—	15·0	—	18	—	12

* Origin of plants used in above Table.

Cross I. 7 × 2.16, lemon × floribunda, yLE × YLE.
Cross I(a). 3 × 42.16, floribunda × lemon, YLE × yLE.
Cross II. 39 × 30, 32 and 34.12, isabellina × floribunda, YlEe × YLE.
Cross II(a). 39 × 25.12 isabellina × kewensis 4n. (Offspring all Yl, E and e.)
Cross III. 27 × 17.20, isabellina × floribunda, ylEe × YLE.
Cross IV. 25 × 29.20, isabellina × yellow, yLE × YyLE.
Cross V. 29 × 3.20, yellow × isabellina, YyLE × YlEe.
Cross VI. 1 × 6.20, isabellina × lemon, Yyle × yLlEe.
Cross VII. Ex 3.20, isabellina self-fertilised, ylEe.
Cross VIII. Ex 16.20, lemon self-fertilised, yLlEe.
Cross IX. F_3 Ex 5 × 17.17, yellow × isabellina, YLE × ylE.

DISCUSSION.

Much of the recent work on polyploidy has been discussed in this *Journal* by C. A. Jørgensen (1928) and we may refer the reader to this paper, more especially to that part dealing with the problem of specific differentiation (pp. 190–205). In the same number of this *Journal* C. D. Darlington described his observations on the cytology of heteroploids in *Prunus*, and discussed the genetical implications many of which bear on the problems met with in *P. kewensis*. We will therefore confine ourselves here as far as possible to certain points in our own work which appear to us to be of peculiar significance.

Primula kewensis was the first example of an allopolyploid formed from an artificial hybrid[1]. Since it was reported by Digby (1912) several other allopolyploids have appeared under observation[2]. In two, viz. the tetraploids of *Raphanus* × *Brassica* (Karpechenko 1927) and of *Digitalis ambigua* × *D. purpurea* (Buxton and Newton 1928) it is clear that the doubling of the chromosome number has been brought about by an incomplete first meiotic division, the anaphasic groups being reunited in a single nucleus which then divides to form dyads with $2n$ chromosomes. This is the process first described by Rosenberg (1917) in *Hieracium*, who later (1927) gave the term "restitution nuclei" to the prematurely formed nuclei with the somatic chromosome number. Similar phenomena were observed by Ljungdahl (1922 and 1924) in *Papaver*, and they would thus appear to be a not uncommon result of disorganisation of the meiotic divisions, at least of the "semiheterotype" described by Rosenberg (1927).

A more frequent source of allopolyploids is perhaps the occasional production by pure diploid species of gametes with the unreduced chromosome

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[1] The term allopolyploid was introduced by Kihara and Ono (1926) to denote polyploids resulting from reduplication of dissimilar sets of chromosomes, as opposed to autopolyploids resulting from reduplication of similar sets, *e.g.* Gregory's *Primula sinensis* (1914), Blakeslee, Belling and Farnham's *Datura Stramonium* (1919) and Gairdner's *Campanula persicifolia* "Telham Beauty," (1926).

[2] Recent additions to those mentioned by Jørgensen are:
    Rubus rusticanus inermis × *Rubus thyrsiger* (Crane and Darlington, 1927),
    Nicotiana Tabacum × *Nicotiana rustica* (Eghis 1927 and Rybin 1927),
    Saxifraga rosacea × *Saxifraga granulata* (Marsden Jones and Turrill 1928).

We may also refer here to Janczewski's case of *Anemone sylvestris* × *A. magellanica* (1889 and 1892) quoted by de Vries in *Die Mutationstheorie*, II, p. 73 and by W. Bateson in *Mendel's Principles of Heredity*, p. 250. Janczewski found that in general the flowers of this hybrid only set one or two seeds, but occasionally completely fertile stems are produced. All the seed, whether from sterile or fertile stems, gave plants like the parent but setting a full complement of seed. From the account it would appear that this hybrid gives rise to tetraploids both somatically and by the production of diploid spores.

number (the process of doubling not as yet observed). The union in hybridisation of unreduced gametes to give tetraploids, or of normal and unreduced gametes to give triploids from which tetraploids are subsequently obtained, is the process by which two recently recorded tetraploid hybrids have arisen, viz. *Rubus rusticanus inermis* × *Rubus thyrsiger* (Crane and Darlington 1927) and *Nicotiana Tabacum* × *N. rustica* (Eghis 1927, and Rybin 1927).

To neither of these categories does *kewensis* 4*n* belong, for it has certainly originated from a doubling of the chromosomes in the soma of the diploid hybrid. It differs also from *Brassica-Raphanus* and *Digitalis ambigua* × *purpurea*, in that the meiotic divisions of the diploid hybrid are perfectly regular. In meiosis of the tetraploid, a quadrivalent chromosome is generally formed, and occasionally two or three; among the bivalents some degree of secondary pairing has also been observed. The formation of quadrivalent chromosomes might be expected to lead to segregation, but their absence would not be an indication that segregation could not occur. In this connection we may compare the chromosome behaviour of *kewensis* 4*n* with that of the two autopolyploids *Datura Stramonium* 4*n* and *Primula sinensis* 4*n* (Belling and Blakeslee 1924). In the former, at late prophase and metaphase, the chromosomes are "as a rule arranged in connected sets of 4 each." But in *Primula sinensis*, quadrivalents are "difficult to demonstrate;...it seems as if the majority of the 48 chromosomes were usually arranged in the tetraploid *Primula* in sets of two pairs each, and rarely 12 such sets may be counted." In *Primula sinensis* and in *Datura* genetical analysis shows that chromosome pairing is free, at least in the chromosomes investigated (Muller 1914, Sverdrup and de Winton unpublished, and Blakeslee, Belling and Farnham, 1923).

The genetical observations indicate that a character of special significance in the group is that of heterostylism. In the parent species long- and homo-styled forms are perfectly self-fertile (*verticillata* exists only in the homo-styled condition). Yet in crosses involving specific differences, the factors concerned in heterostylism play an important part in relation to fertility and viability. This is shown by the following facts:

(*a*) *kewensis* 2*n* as judged by its offspring has been produced from *floribunda* long-styled × *verticillata*. We have made many attempts to raise it from *floribunda* homo-styled × *verticillata* but have not succeeded.

(*b*) From crosses between homo- and long-styled varieties of *floribunda* with *kewensis* 2*n* (heterozygous homo-styled) as the male parent,

the progeny are either long-styled (if the mother was long-styled) or heterozygous homo-styled (if the mother was homo-styled). Thus the effective hybrid gametes in these crosses all carry the long-styled factor introduced into the hybrid by *floribunda*.

(c) From crosses of *kewensis* 4n long-styled, or homo-styled but throwing homo- and long-styled offspring, with varieties of *floribunda*, triploid and tetraploid plants (5) have been raised. Similar crosses made with *kewensis* 4n homo-styled and true breeding have entirely failed to give viable seed. Of the five plants so raised, four were long-styled and one homo-styled, (the homostyle gene derived from the diploid parent). The long-styled plants are highly fertile, the homo-styled plant is sterile as a female but slightly fertile as a male.

It is clear that except in a few of the above crosses, the comparative fertility of legitimate and illegitimate crosses has no direct bearing on the results. Between legitimate and illegitimate crosses the difference is zygotic, all the pollen grains of a heterozygous plant having the same properties in fertilisation, in spite of their different genetical constitution. But in our cases the production of offspring appears to depend on the genetic constitution of the gametes, while in (c) sterility seems to follow from the introduction of the homostyle gene. These observations indicate a lack of compatibility between the homostyle factors of *verticillata* and *floribunda* greater than that between the homo- and long style factors of these species.

Among geneticists the opinion is generally held that allopolyploids such as *kewensis* may be classed as species (Renner 1924, p. 344; Clausen 1926, p. 136 and others). For *kewensis* 4n we can however scarcely claim the rank of a "good" species, even if we take into account only the variability which we ourselves have seen and attempted to describe in these pages, but we have reason to believe that other derivative forms are in existence, although the plant is not widely grown in this country. Further, the experimental evidence points to an increased variability among the derivatives rather than a greater stability than is found in their progenitor. This increase may only be apparent, for a change in genetical constitution involving the loss of certain factors might possibly bring to light other variations. But it may also follow from an increased capacity for interchange between specifically distinct chromosomes in the homologous "set" concerned in the initiation of a variation.

SUMMARY.

1. *Primula kewensis*, the diploid hybrid of *P. floribunda* × *P. verticillata* ($2n = 18$ chromosomes) has been observed to set seed on three occasions since its first production in 1900. Each time its seed has given rise to fertile plants with the tetraploid number of chromosomes (36). In the vegetative cells of one of these fertile inflorescences we have found the tetraploid number of chromosomes, showing that the doubling process takes place in the somatic divisions. We believe this to be the only case known of a sterile (diploid) hybrid giving rise to a fertile tetraploid by somatic doubling of the chromosomes.

2. Meiosis in *kewensis* $2n$ is regular (Digby, 1912). Although the plant does not set seed in the diploid state, its pollen is, in a slight degree, fertile on the parent species *floribunda*. From this cross we have raised 36 plants (an average of 6 per capsule) closely resembling *floribunda* but with traces of *verticillata* characters. We suggest therefore that the sterility of the diploid hybrid is due to non-viability of the greater number of chromosome (or factorial) complements formed in the meiotic divisions. The failure of the hybrid to set seed when fertilised by *floribunda*, although its pollen is in some degree fertile on this species, may be an indication that the male sterility is the result of zygotic non-viability, all bad pollen grains being eliminated from fertilisation, whereas female sterility must necessarily result both from gametic and zygotic non-viability.

3. Meiosis in the tetraploid may be almost as regular as in the diploid, as was shown by Digby. To explain the high degree of fertility and also the comparative constancy of the tetraploid, Winge's hypothesis of intraspecific pairing of the chromosomes is adequate with the provision that occasionally pairing is between unlike chromosomes (interspecific pairing). The assumption made by Farmer and Digby that the doubling was due to transverse fragmentation is shown to be untenable, and their measurements unreliable.

4. Sporadic variation of the tetraploid has frequently been found to be associated with the loss or gain of a chromosome. The descendants of three such chromosome aberrations have been observed for several generations. The characteristic features of these three lines of descent are:

(1) Pale yellow flowers combined with pale foliage and early maturity (type *B*, Plate XI).

(2) Small leaves, a tendency of the leaves to cohere (type *C*, Plate XII).

(3) Heterostylism. The general habit and foliage is also affected (type *D*, Plate XIII).

If the original variation was due solely to the loss or gain of a chromosome, it would be expected that in each of these three families, a form would be represented corresponding to the "balanced tetraploid," *i.e.* resulting from the restoration, by fertilisation, of complete sets of *floribunda* and *verticillata* chromosomes. Nevertheless, we have failed to identify a form common to all three lines of descent. We therefore suggest that the original loss or gain of a chromosome occurred after, and was possibly the result of, interspecific pairing and interchange between chromosomes.

5. Variability in the tetraploid not associated with chromosome aberration occurs in mealiness and leaf shape. Plants differing in mealiness have been bred from, and the results suggest that more than one pair of allelomorphs are involved. They are definitely against an explanation based on substitution of chromosomes within a single set.

7. A plant with 26 chromosomes, derived from a presumed triploid by self-fertilisation, has been investigated[1]. In meiosis it shows 10 bivalents and 6 univalents. It is fertile as a male on *floribunda* and among its descendants are plants in which *verticillata* and *floribunda* characters are combined.

8. Five plants have been obtained from crossing diploid varieties of *floribunda* with tetraploids of type *D*, *i.e.* long-styled or heterozygous for long style. Three of these plants have the triploid, or approximately the triploid number of chromosomes, and two approximately the tetraploid number. In appearance they are alike, with the characters of *floribunda* rather than of the tetraploid or diploid hybrid. Presumably those with approximately tetraploid chromosome numbers arose from gametes of the diploid parent with an unreduced nuclear complement.

APPENDIX.

Classification and description of types.

In the classification of *Primulaceae* adopted by Pax (1888) two sections, the *Floribundae* (now known as *Verticillata*) and the *Auriculas*, are separated from all others by the involute vernation of the young leaves, *Floribundae* being the only Eastern group in which the vernation is not revolute. In this group are included only three species. *Aucheri*, a rare form of which little is known, *floribunda* and *verticillata*. They

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[1] This plant, no. 18/12, was formerly described as a diploid (Pellew and Durham, 1916).

are distributed as follows: *Aucheri* and *verticillata* in S.W. Arabia and Abyssinia, *floribunda* in Afghanistan and the W. Himalayas.

Of *verticillata* Forsk., Professor Bayley Balfour wrote in 1913 (*J.R.H.S.* Vol. xxxix) "Two microforms are recognised, *P. Boveana* Decne, and *P. simensis* Hochst. the latter the form which occurs in Abyssinia. Indeed the plant introduced as *P. verticillata* and figured in the *Botanical Magazine* in 1828 is the microform *P. Boveana* Decne." The plant here figured (*B.M.* t 2842) has flowers smaller than we have ourselves seen in *verticillata*. The limb of the corolla is irregularly crenate and the segments of the calyx deeply toothed. (The last two features appear sporadically but associated in *kewensis* 4*n*, type *D*; we have not observed them in any other form.) The second microform, *simensis*, probably corresponds to the plant figured in 1873 (*B.M.* t 6042), and is there said to have been introduced recently by Messrs Veitch. The flowers of this plant are exceptionally large ("the corolla tube one inch long, limb nearly as much in diameter"); the lobes of the corolla notched, not crenate, and the calyx segments entire.

The strain of *verticillata* grown at this Institution for many years appears to be Forskaol's type; it has small flowers as compared with *simensis*; the calyx segments are entire, and the lobes of the corolla notched, not crenate. But recently we have received plants of another strain cultivated in the garden of Sir William Laurence, which differs slightly from our own; the leaves are broader, less meal is produced, and the leaf bases are more deeply pigmented.

Of *floribunda* Wall. no microforms are recorded, and the plant figured in 1883 (*B.M.* t 6712) appears to be identical with our own strain. In 1925 (*J.R.H.S.*, p. 295) Sir George Watt wrote of the species in its native habitat, saying that at low altitudes the plant becomes "large, more robust, quite glabrous, often mealy, and the bracts foliaceous." We have never observed any decrease in hairness, but in the summer months there is an increase in the amount of secretion from the glandular hairs[1]. It may be that the change in habit of the plant observed by Sir George Watt is a similar but more extreme effect of high temperature.

The isabelline variety of *floribunda* brought out by Messrs Haage and Schmidt in 1897 may be regarded as a microform. It has probably appeared in cultivation. We have seen this variety only in the long-styled form;

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[1] Of the nature of the substance secreted little is known, but it has been suggested by Miss E. Philip Smith that it is an oil. The meal of *verticillata* is readily soluble in cold alcohol, and is therefore not a wax. (Solereder's *Systematic Anatomy of the Dicotyledons*, translated Boodle and Fritsch, I, p. 503.)

it differs from *floribunda* type in the cream colour of the flowers, and also in having longer hairs and paler anthocyanin pigmentation in the stem and leaf bases.

TABLE V.

Flower measurements in millimetres; Pollen measurements in μ
(the pollen in 1 % glycerine solution).
L.S. =long-styled. All others homo-styled.

	Length of corolla tube	Length of style	Height of anthers in tube	Size of pollen grains average	Number of grains measured	
verticillata	25	24	23	27·5	36	
*floribunda	10	6	7	27·5	64	
†*isabellina* **Yl** (L.S.)	10	7	3	19·0	30	
‡*isabellina* **yl** (L.S.)	9	6	2	21·5	30	
kewensis 2n.	20	15	15	—	—	Few good grains
kewensis 4n.						
No. 11^47/27, normal	21	16	15	28·5	16	Few shrivelled grains
No. 11^41/27, normal	20	15	15	27·5	28	Few shrivelled grains
No. 11^39/27, normal	21	17	15	27·5	15	
No. 11^1/27, normal	19	18	15	26·5	30	Many shrivelled grains
Average 2 plants, type *B*	19	14	14	26·5	32	Few shrivelled grains
Average 2 plants, type *B*	19	15	15	27·0	32	Many shrivelled grains
Average 2 plants, type *C*	22	17	16	26·0	32	Many shrivelled grains
Average 2 plants, type *D*	17	15	13	26·5	66	Many shrivelled grains
Average 5 plants, type *D* (L.S.)	14·5	10	6	22·0	96	Many shrivelled grains

  * We have no long-styled *floribunda* available for comparison with the homo-styled plants.
  † This is the variety brought out by Messrs Haage and Schmidt in 1897.
  ‡ This variety is derived from *floribunda* × 18/12.

In the following list the most important differences between the parent species and *kewensis* 2n are shown. In Table V, the size of pollen grains and the relative measurements of the parts of the flower in homo- and long-styled plants of many of the forms investigated are given[1].

P. verticillata	*P. floribunda*	*P. kewensis 2n*
Size		
Peduncles to 1st whorl about 20 cm.	About half that of *verticillata*	About that of *verticillata*
Surface		
Whole plant covered with short glandular hairs from which meal is secreted	Covered with hairs of two kinds: (*a*) short glandular, and (*b*) long, more or less glandular: very little secretion	Covered with short glandular hairs, meal not visible except on the inflorescence where it is plentiful

  [1] In *Primula kewensis* a number of problems await investigation, and it is hoped that other students will take it up as a subject for research. Some of the most interesting forms here described are being cultivated at this Institution. On application seed and plants would be distributed.

Flowers

Pale yellow.	Deep yellow.	Deep yellow.
Surface of petal shiny, papillae rounded.	Surface of petal dull, papillae pointed.	Surface of petal shiny, papillae rounded.
Sweet scented	Scentless	Sweet scented

Leaves

| Obovate, pointed, acutely toothed, smooth | Ovate, more or less pointed, crenately toothed, rugose | Obovate, pointed. Almost crenately toothed, smooth |

Anthocyanin

| Leaf bases pigmented | Leaf bases peduncles and pedicels heavily pigmented | As in *floribunda* |

Habit

| Perennial, long-lived | Perennial, but in our experience seldom lives more than 2 years | Perennial, long-lived |

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PLATE XI

PLATE XIII

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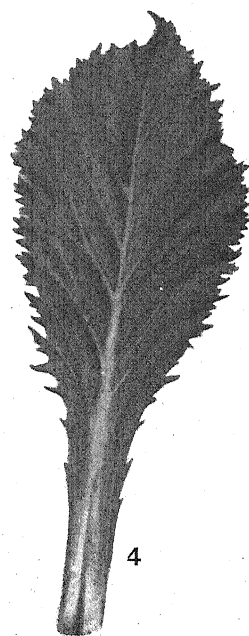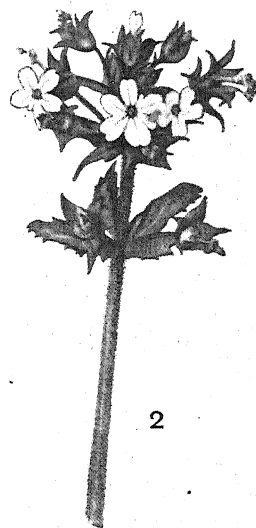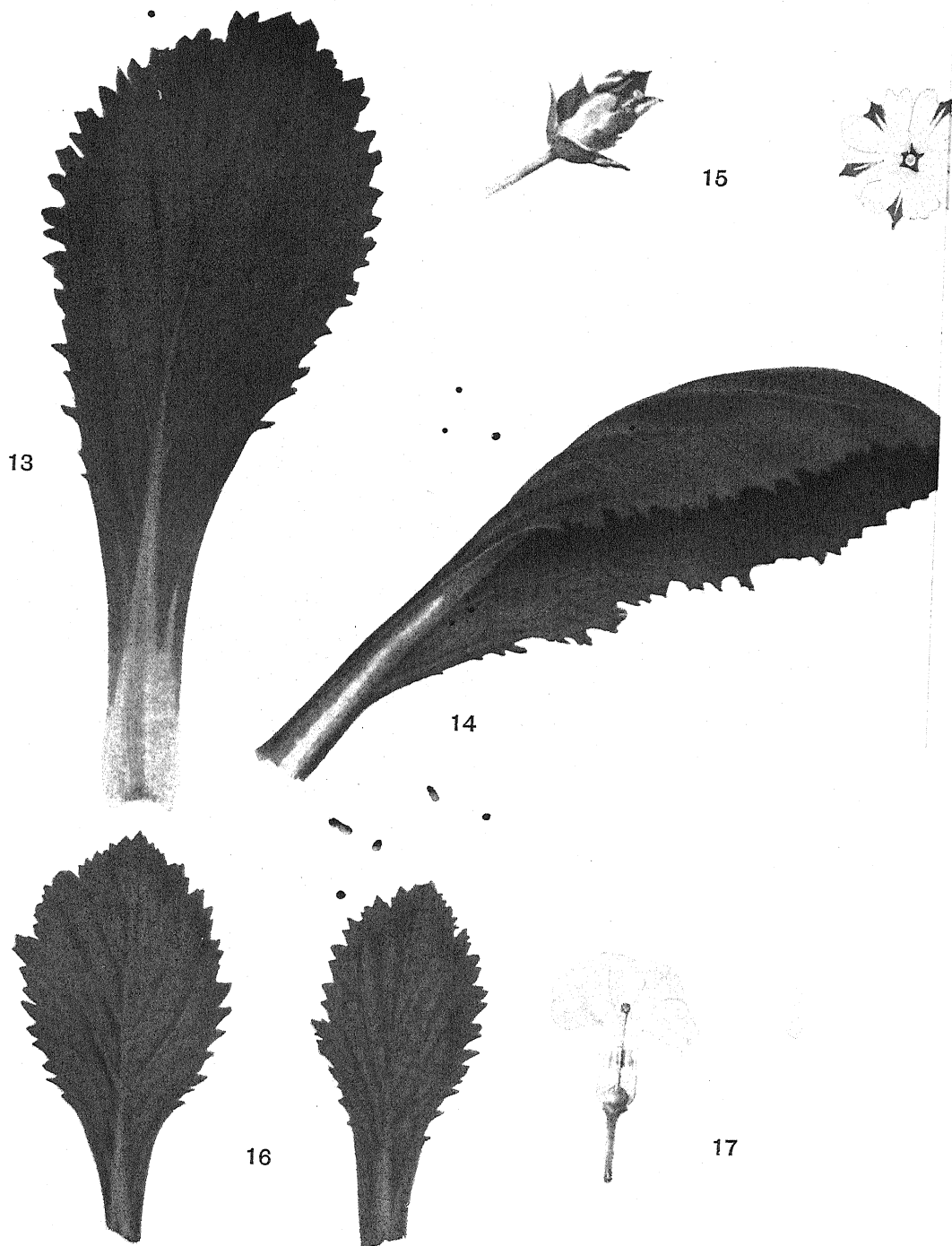
DESCRIPTION OF PLATES XI—XIII.

PLATE XI.

Fig. 1. *P. verticillata*, inflorescence.
Fig. 2. *P. floribunda*, inflorescence.
Fig. 3. Type *B*, 34-chromosome plant. (This is represented too dark a shade of green.)
Fig. 4. Leaf of 19/25, type *B*, 35-chromosome plant.

PLATE XII. TYPE *C*.

Fig. 5. Leaf of 81/26, 36 chromosomes.
Figs. 6–8. 34-chromosome plants.
Fig. 6. Sterile, upright "spiral" form, 48A/26.
Fig. 7. Sterile, flat "spiral" form in family 73/27.
Fig. 8. Sterile, small, in family 77/27.
Fig. 9. Flower of 81/26, 36 chromosomes.
Fig. 10. Flower of 48^1/26, 35 chromosomes.
Fig. 11. Flower of sterile "spiral," 48^{11}/26, 34 chromosomes.
Fig. 12. Sectorial chimaera, 76^1/27.

PLATE XIII. TYPE *D*.

Fig. 13. Leaf of 59/27, long-styled, 37 chromosomes.
Fig. 14. Leaf of 90^3/27, long-styled.
Fig. 15. Flowers of 90^3/27.
Fig. 16. Leaves of two small leaved long-styled plants from 52/26, self-fertilised.
Fig. 17. Sections of flowers of long and homo-styled plants.

Drawn by Mr C. E. Osterstock.